Third Report to the Royal Society Water Research Committee.

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PART I.

"Further Experiments on the Action of Light on *Bacillus anthracis*, and on the Bacteria of the Thames." By H. MARSHALL WARD, D.Sc., F.R.S., F.L.S., F.R.H.S., Professor of Botany, Cooper's Hill.

In a previous Report to the Committee, I have shown that the action of light on bacteria is not only very definite, and much more pronounced than had hitherto been supposed, but that it has an importance in its bearing on the question of the destruction of these organisms in the water of rivers, ponds, &c., vastly greater than had ever been suspected.

In this Report I offer some of the results of long continuous investigations into the details of this bactericidal action of light—both solar and electric—and into the bacterial flora of the River Thames, as studied at a point where it flows below Cooper's Hill.

The reader may be referred to the previous Reports* for details as to the methods of investigation employed, and as to the chief results obtained by exposing the spores of *Bacillus anthracis* to the direct action of undecomposed solar light; he will also find further details in two papers presented to the Royal Society in 1892 and 1893† regarding this matter, and regarding preliminary investigations into the action of solar light which has been passed through various absorbent media.

Special attention may be directed to pp. 23—34 of the 'Proceedings,' vol. 53, and the following experimental results may in the first place be taken as supplementing those there published.

* 'Proc. Roy. Soc.,' vols. 51 and 53.

† 'Proc. Roy. Soc.,' vol. 52, pp. 393—400; and vol. 53, pp. 23—44.

Table A.—Experiments with Spores and Media separate.

Number of plate.	Nature of plate.	When made.	When exposed.	Number of hours sunlight.	When put to incubate.	Number of days incubated.	Results, indicating the figure or letter used.	Remarks.
D (1)	Agar only	Feb. 12	Feb. 12 and 13	5 (partly fitful)	..	8	No trace whatever of the letter Y, the spores germinated evenly all over	In these cases the *exposed* slab of agar was placed on a dry film of *unexposed* spores before incubation.
E (1)	,,	..	,,	,,	..	5	,,	
F (1)	Spores only	..	,,	,,	..	5	No results beyond a dim "ghost" of a letter, owing to overgrowth	I made the mistake of adding a little water after putting a slab of unexposed agar on the spores; the water ran in and flooded the plate.
H (1)	,,	..	Feb. 13	3 (fitful)	..	8	Good letter U, but developed very slowly	The extreme slowness of development here was due to my using large slabs of agar, and therefore obstructing aeration.
I (1)	,,	..	,,	,,	..	8	Good letter T, very slow	
J (1)	Agar only	..	,,	,,	..	8	No trace of letter C.	
A	Spores only	Feb. 18	Feb. 19	6 (reflected)	Feb. 20	1	Excellent and sharp W	
B	,,	,,	,,	,,	lost			
C	Agar only	,,	,,	,,	Feb. 20	4	No trace of letter Z.	
D	,,	,,	,,	,,	,,	4	,,	
E	Spores only	,,	Feb. 19 and 20	5*	Feb. 21	1	Good sharp E........	* Behind glass screen (ordinary 10).
F	,,	,,	,,	5	,,	2	Letter X distinct......	Developed slowly at first, but sharp and clear afterwards.
G	,,	,,	,,	5†	,,	1	Sharp letter H........	† Behind blue glass screen (Blue 1).

H	,,	,,	Feb. 25	5‡	Feb. 25	1	Perfectly clear E......	‡ Behind an alum screen $\frac{1}{3}$ in. thick. The interval between 18th—25th February was dull and cold.
D	Spores and agar block	Mar. 20	Mar. 21	$3\frac{1}{4}$	Mar. 21	17 hours	The letters (B and C) visible at 9 A.M. on 22nd, and *sharp* at 5 P.M.	Very hot, brilliant sun and blue sky. Excellent letters on 23rd, but not all the spores killed.
F (*a*)	Spores only	,,	,,	$2\frac{3}{4}$	,,	6 days	Traces, 5 P.M. on 22nd, of T	No further results. No trace of T after.
F (*b*)	Spores and agar block	,,	,,	,,	,,	6 ,,	Traces of ✠ at 5 P.M. on 22nd	Not all killed, but good cross.
G	Spores only	,,	,,	3	,,	$23\frac{1}{2}$ hours	Just visible, 3 P.M. 22nd	No further result. No trace on 24th; kept till 27th.
H	Spores and agar blocks	,,	,,	3	,,	6 days	Cleared area only......	Obliterated later.

§ I.

Before proceeding to the results obtained by exposure behind coloured screens, I select the following series of further experiments, which confirm some of my previous statements, referred to above (Table A).

It is worth remark that these exposures were made in February, at a time when the temperature was low, and the sunlight, though bright, of an intensity far below that obtainable in the summer. The methods of preparing the agar plates and films of dried spores, have already been described, that is to say, in experiments numbered D (1), E (1), J (1), C and D, a plate of sterile agar was exposed, in each case behind a stencil plate, and, after exposure, was laid flat on a film of dried unexposed spores of *B. anthracis*, whereas in the cases marked otherwise it was the film of spores which was thus exposed, and a sterile plate of unexposed agar then placed on the film. Incubation then decided whether the light had produced any effect, the results being given in the table.

These results fully confirm those obtained previously, and show that the action of the light is direct on the spores, and not on the food material—in this case agar—in which the spores are suspended. That the slow development in the cases marked H (1) and I (1) was due to deficient aeration—possibly in part also to the fitful sunshine to which the plates were exposed—is borne out by the following experiments. Three stout glass tubes were selected, sterilised, and charged each with about 5 c.c. of bouillon, in which was distributed a small loop-full of the spores of *B. anthracis.* Each tube was about 6 in. long, and, after charging, was plugged with sterilised cotton wool, the plug being pushed 2 in. into the tube. Each tube was then drawn out to a point, exhausted of air, and the end sealed in a flame. The vacuum tubes were kept thus sealed until the following day, when two of them were broken at the tips, in a flame, to let in air; the other remained sealed.

The sealed tube, and one of the now unsealed tubes, were then exposed all day to a bright sun, while the third (unsealed) tube was wrapped in tin-foil and black paper, and placed side by side with the others, thus protected from the light. After six or seven hours' exposure, the tip of the still sealed tube was also broken, and all three placed in the incubator at 22° C.

In forty-eight hours the covered tube and the exposed sealed tube were equally and copiously turbid, with a vigorous growth of the bacillus; the exposed *unsealed* tube showed the faintest traces only of this turbidity.

The inference is obvious. Exposure to sunlight *in vacuo* results in no perceptible retardation or destruction of the bacillus, whereas if

exposed in contact with air nearly all the spores are killed in the time given. As will be shown later, the thickness of the glass of which the tubes were composed is no doubt an important factor, and probably *all* the spores in the exposed unsealed tube would have been killed had the glass been thinner, as they certainly could have been by a longer exposure.*

§ II.

The following series of experiments were carried out during February and March of this year (1893) to obtain some information as to the time of exposure necessary to kill the spores of *Bacillus anthracis*, for I found it desirable to make myself as well acquainted as possible with the power of the solar rays in this respect, in order to utilise the experience in succeeding work. As the table shows, I also tried comparisons between the action of direct and that of reflected sunlight. As the experiments proceeded, it turned out that several difficulties have to be met in attempting to compare the action on two or more different plates exposed side by side.

It seems impossible to ensure absolute similarity between any two plates, for the following reasons:—

1. The difficulty of distributing the spores in equal quantities, and at equal distances apart in the agar. The best results were obtained by pouring the agar on all the plates from one large tube, in which the infected melted agar is thoroughly shaken; but even then it was impossible to be sure that the agar film in each plate was of equal thickness. Of course practice and experience enable one to pour approximately the same quantity of the agar into each plate, but this does not entirely overcome the difficulty.

2. Even very careful selection from a large number of the Petri's dishes does not secure that each dish used shall have a perfectly plane glass face (to be exposed), of equal thickness, and identical in its properties towards the light. Here, again, therefore, one had to be satisfied with as close approximations as possible.

It was owing to these difficulties that I hit upon the device of employing one plate with several square or circular "windows" cut in its covering, at equal distances apart. After exposing all the "windows" for, say, half an hour, one was then covered; after a further exposure of half an hour, a second one was covered, and so forth (or, conversely, the windows uncovered in succession), as in the cases marked I*a* to I*d*, and 5*a* to 5*d*, &c., on Table B.

But another difficulty now made itself evident, namely, that as the intensity of the solar light may vary considerably from time to time not only owing to altitude, but also to differences in the atmosphere,

* It may be pointed out that these results confirm those of Roux, 'Ann. Past. Inst.,' 1887.

Table B.—Experiments with Spores in Agar, without Screens.

Number of plate.	Nature of plate.	Date made.	When exposed.	Number of hours sunshine.	When put to incubate.	Number of days incubated.	Results.	Remarks.
A (1)	Agar-spores	Feb. 12	Feb. 12	3	Feb. 12	3	No distinct letter E came out	The sunlight fitful between clouds.
B (2)	,,	,,	Feb. 12	2	,,	3	Good letter Z, though the plate had slipped	Sunlight interrupted by clouds.
T	,,	Feb. 28	Feb. 28	4	Feb. 28	4	No trace of germination anywhere on plates	
7	,,	Mar. 4	Mar. 5	1	Mar. 5	6	Only half-a-dozen colonies	Direct sunlight.
8	,,	,,	,,	1	,,	6	A dozen colonies	Reflected sunlight.
9	,,	,,	,,	4	,,	6	Only a couple of colonies.	Direct sunlight. } Only the first hour direct sun, the other three bright, diffused, cloudy.
10	,,	,,	,,	4	,,	6	A few colonies only....	Reflected sunlight.
13	,,	Mar. 7	Mar. 7	2	Mar. 7	2	Sharp B, but 20—30 colonies on it	Reflected sunlight. } Cloudy, with hot glare, & gleams of bright sun.
14	,,	,,	,,	2	,,	2	Good C, but less sharp edges; about same number of colonies	Direct sunlight.
I*a*	,,	Mar. 10	Mar. 10	$\frac{1}{2}$	Mar. 10	2	Merest ghost of square	Successive squares on same plate and direct light.
I*b*	,,	,,	,,	1	,,	2	Good square, but not *all* killed	
I*c*	,,	,,	,,	$1\frac{1}{2}$	,,	2	Square quite clear, and sharp	
I*d*	,,	,,	,,	2	,,	2	Square quite clear, and sharp	

IV*b*	,,	,,	,,	1	,,	1	Cross visible in 20 hours, and very sharp in 45 hours	A ✠ cut in plate over mirror.
VII*a*	,,	Mar. 11	Mar. 11	2	Mar. 11	6	No trace of germination	Letters B, C on same plate. C exposed three hours and B only two to direct sun. Internal reflection (?) inhibited.
VII*b*	,,	,,	,,	3	,,	6	Ditto	
VIII	,,	,,	,,	3	,,	6	Centre clear, and in a somewhat vague Y-shape at last	Direct sun. Letter Y.
IX	,,	,,	,,	3	,,	2	Sharp X in 40 hours	Reflect sun. The X is nearly clear—only about a dozen colonies on it—on third day.

Table B—*continued.*

Number of plate.	Nature of plate.	Date made.	Date exposed.	Kind of exposure.	Hours' sun.	Into incubator.	Hours incubated at 25° C.	Results.	Remarks.
5*a*	..	Mar. 13	Mar. 13	Direct	1. 45—4. 15	4. 30 P.M. Mar. 13	40 hours	Window distinct, but only about half the spores killed.	A four-windowed plate. Sun obscured by cloud, and very dull after 2.45 P.M.
5*b*	..	,,	,,	,,	2. 15—4. 15	,,	,,	Window *just* perceptible	
5*c*	..	,,	,,	,,	2. 45—4. 15	,,	4 days	No trace: germinated all over	
5*d*	..	,,	,,	,,	3. 15—4. 15	,,	,,		
XVIII	Ag. sp.	Mar. 27	Mar. 27	,,	12. 15—3. 15	3. 15 P.M. Mar. 27	,,	In 18 hours = excellent Y, rapidly sharpening up to 5 P.M. On 29th best letter seen. Nearly cleared	Very hot sun, hazy first hour, then brilliant.
XX A	,,	,,	,,	,,	12. 30—2. 30	3. 45 on Mar. 27	,,	Could detect little or no difference between A, B, D, and E, and C = spoilt owing to contact with lid.	
XX B	,,	,,	,,	,,	12. 30 —3. 45	,,	,,		
XX C	,,	,,	,,	,,	12. 30—1. 30	,,	,,		
XX D	,,	,,	,,	,,	12. 30—3. 45	,,	,,		
XX E	,,	,,	,,	,,	12. 30—3. 30	,,	,,		

and to clouds passing, &c., &c., it seemed utterly hopeless to expect the accurately comparative results required.

Taking all these drawbacks into consideration, the Table B nevertheless shows some significant and instructive facts.

The experiment denoted I*a*, for instance, shows that, even in March, the solar action can be detected clearly after so short an exposure as half an hour to an hour, while exposures of $1\frac{1}{2}$ to 2 hours resulted in sharp, clear figures.

After a large number of these comparative trials, however, I concluded (1) that while it seems impossible to overcome all the difficulties, and to express the nature of the exposure in words, the general impression gathered was that on certain bright, sunny days in the spring—days when the sky is blue and cloudless, and the air peculiarly clear, the bactericidal power of the direct or reflected solar rays is very great—much greater than has been supposed. A very slight amount of haze makes a vast difference in the times of exposure (*e.g.*, Cases A (1), and B (1), where a much better result was obtained in two hours in the one case than with three hours in the other); (2) that very long exposures are necessary if the sky is overcast with clouds, even though the light is otherwise bright. In other words, the direct rays of the sun are needed for the purpose of rapid action; (3) with solar light, direct from the sun, very little if any difference can be detected between exposures where the rays fall *directly* on the plate, and where they are once reflected from a thin plane glass mirror silvered at the back.*

Summed up in the shortest terms, the conditions of exposure are practically the same as those required in ordinary photography, the chief difference being that the duration of the exposures amounts roughly to hours or half hours in the cases under consideration, instead of minutes or seconds, as in quick plate photography. All this, of course, points to the blue end of the spectrum as the effective one, a conclusion which is abundantly justified, and, in fact, fully proved in the sequel, and by my experiments with the spectrum since published.†

§ III.

In the following sets of exposures (Table C), I employed quartz, instead of glass, as a covering to the Petri's plates, so that, except in the cases 12A, 12B, and 12C, the light traversed no glass before reaching the spore-laden film.

The results show little additional information, excepting that in the cases 12D and 12E it was interesting to find that the action was approximately as pronounced after one hour of exposure as after

* This remark refers particularly to this kind of exposure.

† 'Proc. Roy. Soc.,' vol. 54, p. 472 (Abstract).

Table C.—Experiments with Quartz.

Number of plate.	Letter, &c., used.	Date made.	Date exposed.	Time of exposure.	Nature of exposure.	Date put to incubate.	Period of incubation.	Time when letter, &c., first seen.	Screen, if any.	Results.	Remarks.
11.1	□	Mar. 16	Mar. 17	9.30—10	Direct sun	12 noon, Mar. 17	$28\frac{1}{2}$ h.	4.30, Mar. 18	None, except the quartz	All the squares showed action.	Brilliant sun and blue sky, but windy and driving clouds. Plate with 5 square windows open on the quartz. All five squares visible at 4.30 on 18th. On the 19th the order in rank of clearness = 4 and 5 clearest : 3 : 2 and 1.
11.2	,,	,,	,,	9.30—10.30	,,	,,	,,	,,	,,		
11.3	,,	,,	,,	9.30—11	,,	,,	,,	,,	,,		
11.4	,,	,,	,,	9.30—11.30	,,	,,	,,	,,	,,		
11.5	,,	,,	,,	9.30—12	,,	,,	,,	,,	,,		
12 A	○	Mar. 17	Mar. 17	1.30—2.30	Reflect sun	4 P.M., Mar. 17	40 h.	9 A.M., Mar. 19	Ordy. 9	All the circular windows showed action.	Plate with 5 circular windows, of which A, B, and C had screens superposed on the quartz. Windy, and more clouds than in morning, but brilliant blue and sun at intervals. On 19th D and E (the quartz without other screen) were clearest, B next, and A least clear.
12 B	,,	,,	,,	1.30—4.0	,,	,,	,,	,,	,,		
12 C	,,	,,	,,	1.30—4.0	,,	,,	,,	,,	Blue 1		
12 D	,,	,,	,,	1.30—2.30	,,	,,	,,	,,	None		
12 E	,,	,,	,,	1.30—4.0	,,	,,	,,	,,	,,		
IX A	⊙	Mar. 24	Mar. 25	10.30—2.30	Direct sun	4 P.M., Mar. 25	3 days	10 A.M., Mar. 26	Quartz	All the circular windows showed action.	Hot sun, but very hazy. The sharpness and clearness of the action were directly proportional to the length of exposure, *i.e.*, C was least cleared, and B most cleared, and so on.
IX B	,,	,,	,,	10.30—3.30	,,	,,	,,	,,	,,		
IX C	,,	,,	,,	10.30—11.30	,,	,,	,,	,,	,,		
IX D	,,	,,	,,	10.30—1.30	,,	,,	,,	,,	,,		
IX E	,,	,,	,,	10.30—12.30	,,	,,	,,	,,	,,		

two and a half hours, and distinctly more so in both cases than where glass was employed in addition.

§ IV.

The following series of experiments (Table D) were made in continuation of the foregoing, and the description of glass screen employed (3rd column) refers to the table on pp. 328—329.

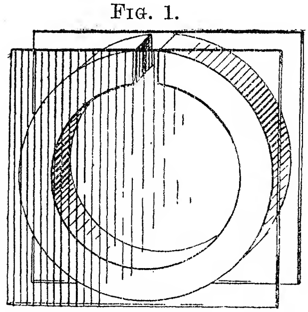
The chief feature of novelty is that I here used the *same* plate for different screens, as follows. A thick, opaque cardboard screen was prepared as large as the plates, and this screen perforated with four or five circular or square windows. Over each hole a piece of the coloured or other glass to be employed was then cemented, and the whole held in contact with the plate to be exposed, by elastic bands.

The advantage of this proceeding was that I could check the preceding results, to see if any erroneous conclusion had resulted from my using different plates.

§ V.

In order to investigate more in detail the action of the decomposed sunlight on the spores, I made a series of glass screens of the nature of water cells, or reservoirs, as follows. A number of circular, flat, indiarubber packing rings, about a quarter of an inch thick and three inches internal diameter were obtained, and a small piece cut out of each; then a thin, plain piece of glass was cemented to each side of the now incomplete ring, thus forming a reservoir with two large parallel glass ends, the sides being formed of indiarubber. I found that by carefully cementing the glass with gold size, it held very well, at least for two or three experiments, and could easily be recemented if necessary.

Fig. 1.



These glass cells were filled with the coloured transparent solutions to be referred to (see Table E), and then placed over the exposed letter on the prepared plate as described in my previous paper (' Proc. Roy. Soc.,' vol. 52, p. 393), being held in position by clips

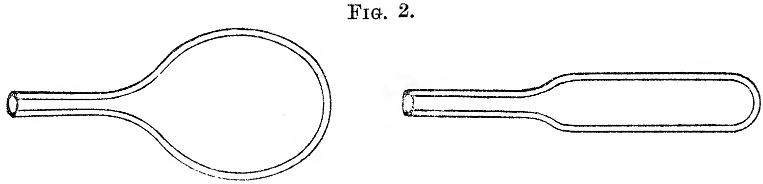
Table D.—Experiments behind other Screens.

Number of Plate.	Date made.	Nature of Screen.	Date exposed.	Time of exposure.	Nature of exposure.	Date put to incubate.	Period incubated at 25° C.	Time letter, &c., first seen.	Results.	Remarks.
6 1	Mar. 13	Ordy. 9	Mar. 13	1.45 to 4.15 P.M.	Direct	4.30 P.M. Mar. 13	40 h.	40 h.	Squares visible, but by no means all killed.	This was a 4-windowed plate arranged so that 2 were covered with glass (ordinary 9) and 2 with quartz.
6 2	,,	Quartz	,,	,,	,,	,,	40 h.	40 h.	Squares possibly a trifle clearer.	
XIV 1	Mar. 27	Blue 7	Mar. 27	12—3	,,	3 P.M. Mar. 27	..	18 h.	On 29th, nearly, but not quite cleared.	Very hot sun, hazy first hour, then brilliant. The plate was covered with black card excepting 5 square holes on which the screens were placed. No trace of action under orange, ruby, or olive glasses.
XIV 2	,,	Orange	,,	,,	,,	,,		..	.. ..	
XIV 3	,,	Ruby 5	,,	,,	,,	,,		..	.. ..	
XIV 4	,,	Olive	,,	,,	,,	,,		..	.. ..	
XIV 5	,,	Ordy. 10	,,	,,	,,	,,		18 h.	Somewhat more cleared than No. 1.	
XV A	,,	Blue 1	,,	,,	,,	,,	..	18 h.	Very sharp and clear square.	An exactly similar plate to the last, and exposed side by side.
XV B	,,	Violet	,,	,,	,,	,,		..	No trace of action at any stage.	
XV C	,,	Ruby 3	,,	,,	,,	,,		..		
XV D	,,	Green	,,	,,	,,	,,		..		
XV E	,,	Olive	,,	,,	,,	,,		..		
XIX	,,	Blue 7	,,	12.15 to 3.15 P.M., Mar. 27	3.15 P.M.	,,		18 h.	Capital sharp X.	

or elastic bands, and the whole then so fixed that the sunlight had to traverse the coloured or other fluid before reaching the agar film in which the spores were embedded. It will, of course, be noticed that the light here traverses three plates of glass as well as the solution of the screen before impinging on the spores in the film, a fact of importance.

I have summed up the characters and chief properties of the solutions employed in the following Table E, and need not, therefore, describe them in detail here. In all cases, excepting Nos. 3 and 5, the medium employed for solution was water; in these exceptional cases, where carbon bisulphide and alcohol were used, it was necessary to have screens devoid of cement. These were met with in the form of certain small glass flasks, shaped like brandy flasks or scent flasks, with a long neck and flat sides; they are used on the Continent for sealing up cultures of bacteria.

FIG. 2.



The chief objection to their use is that the flat sides are apt to be slightly uneven in thickness on the internal face; by carefully selecting from a large number, however, I was able to secure several very good screens of this description.* The liquid is, of course, bottled in the usual way, and the neck secured with a good cork.

§ VI.

The following Table F summarises the results of a number of exposures behind these screens of coloured and other absorbent media, with particulars as to the dates of exposure, number of hours, insolation, and incubation, and other factors worth extracting from the notes.

It will be observed that I here confined my experiments entirely to the spores of *Bacillus anthracis*. I did this because it became more and more evident that until I had obtained all the information possible about some one species—the factor most constant in this long series of slight variables—it would be difficult to value the importance of specific differences later. Experience has thoroughly confirmed the justice of this conclusion.

* In later experiments I have had the side ground out flat, and glass or quartz plates cemented on.

Table E.—Properties of the Screens Employed.

Number.	Colour.	Composition.	Light transmitted.	Rays absorbed.	Remarks.
1	Blue-violet	Ammoniacal cupric oxide	All blue-violet, from line *b* in green	Red-yellow, and part of green	A thick screen of strong solution cuts out almost all but the violet.
2	Blue-green	Prussian blue in oxalic acid	Green and blue	Part of violet, and red-yellow	
3	Purple	Iodine in carbon disulphide	Lower red and deep violet	Red-orange to violet	This superposed on amm. cupric oxide gives a screen opaque to everything below the deepest violet.]
4	Green	Methylene blue and picric acid	Lowest red and whole of green, including traces of yellow- and blue-green	Red-orange, and most of yellow; nearly all beyond green	
	Green	Chlorophyll in alcohol	Most of the red, all the orange, yellow, and green, and a little blue-green	All blue-violet, except a trace beyond green. A deep band in red, and a very faint one in green	These alcoholic chlorophyll solutions rapidly oxidise in sunlight, and become olive. Spectroscopically, the oxidised solutions let through more and more blue, till at last only violet is cut out.
6	Yellow	Picric acid......	All red to green, and a little blue-green	All blue-violet beyond beginning of blue	
7	Yellow	K. chromate (concentrated)	All red to blue, near middle of F.G.	All blue-violet beyond midway between F and G	
8	Yellow	K. chromate (dilute)	All red to blue beyond G.	Violet only, or nearly so	

9	Orange	K. bichromate ..	All red-yellow to *b* in green	All blue-violet beyond *b*	
10	Cherry-red	Eosin in water ..	All red-orange and yellow to near D	All green to violet beyond D	
11	Fluorescent	Sulphate of quinine	All visible rays......	Ultra-violet and about half violet	
12	Colourless	Alum in water ..	All visible rays......	Infra-red only	
13	Crimson	Strong fuchsin in water	Red and a little orange	All beyond red, orange	
14	Lake	Dilute fuchsin in water	Red-orange, and the violet	All from between C and D to near G	By superposing such a screen on one of amm. cupric oxide, all but the violet end can be cut out.
15	Fluorescent	Æsculin (alkaline)	Red, &c., up to half way between F and G in the blue	Cuts off all violet, and some blue	
16	Fluorescent	Æsculin + quinine sulph.	Lets a trifle more blue through	Cuts off a little less blue, but ultra-violet	
17	Colourless	Water	All visible rays......	Infra-red to some extent	

Table F.—Exposures behind

Number of plate.	Date made.	Screen employed.	Date exposed.	Kind of exposure.	Number of hours sunshine.	Date put into incubator.
C (1)	Feb. 12	Quinine sulphate	Feb. 12 and 13	Direct........	5	Feb. 13
I	,, 20	Quinine sulphate	Feb. 25	3 h. reflected: 2 h. direct	5	,, 25
J	,,	Iodine in CS_2..	,,	,,	5	,,
K	,,	Prussian blue and oxalic acid	,,	,,	5	,,
L	,,	Am. cu. oxide..	,,	Reflected.....	5	,,
M	Feb. 25	Weak K. chromate	Feb. 25 and 26	,, ...	3	Feb. 27
N	,,	Strong potass-chromate	,,	,, ...	3	,,
O	,,	Picric acid	,,	2 h. reflected: 1 h. direct	3	,,
P	,,	Methylene blue and picric acid	,,	,,	3	,,
Q	,,	Am. cu. oxide..	Feb. 26 and 28	3 h. reflected: 2 h. direct	5	Feb. 28
R	,,	*Chlorophyll...	,,	,,	5	,,
S	,,	Iodine in CS_2..	,,	,,	5	,,
U	Feb. 28	Weak K. chromate	Feb. 28	Reflected.....	4	,,
V	,,	Strong potass-chromate	,,	,, ...	4	,,
W	,,	*Chlorophyll...	,,	,, ...	4	,,
X	,,	Eosin.........	,,	Direct........	3	,,
Y	,,	Quinine sulphate	,,	,, ...	3	,,
1	Mar. 4	Strong potass-chromate	Mar. 4	Reflected.....	2	Mar. 4
2	,,	Weak potass-chromate	,,	,, ...	2	,,
3	,,	Iod. + CS_2....	,,	,, ...	2	,,
4	,,	Eosin.........	,,	,, ...	2	,,
5	,,	Strong fuchsin	Mar. 5	Direct........	3	Mar. 5
6	,,	Dilute fuchsin		,, ...	3	,,

Bottle Screens, Coloured, &c.

Number of days incubated.	Results.	Remarks.
5	Good letter W	The first three days showed powerful inhibition-effects, and no letter was visible till fourth day: then sharp and clear.
6	Nothing appeared on the plate till the third day, and then only about 200 colonies at the extreme margin. No letter in six days	From 20th to 25th the weather was dull and cold. Temperature of plates averaged 6° C., and none had germinated on morning of 25th, when we had brilliant hot sunshine.
6	No letter in six days	
6	No trace of germination anywhere on the plate, except extreme margin	
6	Excellent sharp letter N	
1	Letter T visible after eighteen hours incubation, but not *sharp*	The letter after three days was not very sharp, since the colonies around were large and not very numerous, and about six or eight were seen on the insolated area.
4	No letter C. Germination took place equally all over the plate	
4	Letter Y feebly visible after twenty-four hours	Even on the fourth day the contrast was not sharp, the surrounding colonies being so few and so large.
4	No letter X. Germination equal all over plate	Closer inspection showed a faint "ghost" of letter X on third day.
3	Sharp letter Z.	
3	No trace of letter	*The chlorophyll at first (Feb. 26) blocked out all the blue-violet, and had bands in red and green; but during the last two hours (Feb. 28) it only cut off violet, and had feeble bands in red and green. Colour olive, in place of deep blue-green.
3	Extremely faint B.	
3	No letter.	
3	No trace of letter.	
3	" "	*The chlorophyll = deepest solution; total absorption from *b* onwards, and deep broad bands in red and green.
3	" "	
3	Faint letter C.	
7	No trace of letter	Temperature rather high. Sun very bright and hot, but interrupted by clouds occasionally. The exposures began before 2 P.M., and ended just after 4 P.M., and two hours expresses the maximum of sunlight. All were over plane mirrors, carefully adjusted. The experiment was of little value. I had probably not used a sufficiently large charge of spores.
7	" "	
7	" "	
7	" "	
6	" "	
6	Very faint letter X	These dilute fuchsin screens need careful watching. The colouring matter gathers into flocks in time, and lets much more light through.

Table F.—Exposures behind

Number of plate.	Date made.	Screen employed.	Date exposed.	Kind of exposure.	Number of hours sunshine.	Date put into incubator.
11	Mar. 4	Strong chlorophyll	Mar. 5	Direct........	3	Mar. 5
12	„	Strong potass-chromate	„	„	3	„
15	Mar. 7	Dilute* fuchsin	Mar. 7	„	2	Mar. 7
16	„	Strong* „	„	„	2	„
17	„	Æsculin + quinine	„	Reflected.....	2	„
18	„	Æsculin.......	„	„	2	„
19	„	Potass-chromate* (strong)	„	Directed......	2	„
20	„	Quinine.......	„	Reflected.....	2	„
III*a*	Mar. 10	Iod. + CS_2....	Mar. 10	Direct........	1	Mar. 10
III*b*	„	„	„	„	2	„
III*c*	„	Strong potass-chromate	„	„	1	„
III*d*	„	„	„	„	2	„
IV	„	Quinine.......	„	Reflected.....	2	„
V	„	Æsculin + quinine	„	„	2	„
VI	„	Æsculin.......	„	„	2	„
X*a*	Mar. 11	Chlorophyll....	Mar. 11	„	2	Mar. 11
X*b*	„	$CuSO_4$........	„	„	2	„
7	Mar. 13	Iod. + CS_2....	Mar. 13	Direct........	$2\frac{1}{2}$	Mar. 13
9*a*	„ 14	„	„ 15, 16, and 17	„	10—12	„ 17
9*b*	„	No screen	„	„	„	„
B 1	Mar. 21	„	Mar. 21	„	2	Mar. 21
B 2	„	CS_2 + Iod.....	„	„	2	„
C	„	Chlorophyll....	„	„	3	„
XVI	Mar. 27	KKS	Mar. 27	„	3	Mar. 27, 3 P.M.
XVII	„	Water	„	„	3	„

Bottle Screens, Coloured, &c.—*continued.*

Number of days incubated.	Results.	Remarks.
6	No trace of letter.	The chlorophyll much oxidised and olive coloured at end, but still cut out all the blue.
6	,, ,, ...	
2	Good X, but by no means all killed	*Plane screen. The dilute fuchsin lets red — yellow and trace of green through, and then blocks up to one-third between F and G. Then lets a considerable proportion of blue-violet through.
4	No trace. Plate evenly covered all over	
1	Extremely faint "ghost" of Z after twenty-four hours, and invisible after forty-eight hours.	
4	No trace. Germination all over	
4	,, ,, ,,	*Plane screen. This chromate is reciprocal to dilute fuchsin.
4	,, ,, ,,	
7	No germination till second day. No letter	Same plate, screens, &c. Successive windows opened. Bottle screen. *Powerful* inhibition on the CS_2 side, and no germination there at all at first. Faint "ghost" after twenty-five hours on chromate side, but obliterated later.
7	No germination till third day. Very faint and transient letter later	
7	Active germination and traces of figures but transient only	
7		
2	Letter T visible in twenty hours, but not all killed.	
1	Letter X visible in seventeen hours, and sharpening up in twenty hours	Over same mirror. Both X and Z gradually obliterated next day—*i.e.*, spores not killed, only retarded.
1	Z visible, &c., *pari passu* with latter	
6	Germination equal all over plate. No letter	Extremely good sun and blue sky.
6	Good letter H.	
4	Germinated evenly all over . . .	Exposed 1.45 to 4.15. Incubated at 25° C.
$16\frac{1}{2}$ h.	No letter appeared.	One screened; the other not. Same plate, &c.
,,	C out sharp, but diffused	
26 h.	Faint N, cleared by 24th, but bad outline	Same plate. H screened. N not. Very hot brilliant sun and blue sky. Kept till 25th. N clearest, but bad.
42 h.	Extremely ill-defined, and never so good on H	
6 d.	Germinated evenly all over.	
. .	Much clearance over a shield-shaped area in eighteen hours, but no clear U	Very hot sun, hazy first hour, then brilliant.
. .	Very sharp and clear T.	

If we now look at the results tabulated above, it is seen that the solar action is evident, though feeble, through dilute fuchsin, æsculin and quinine, and picric acid; while no trace of action occurred through potassium chromate, chlorophyll, eosin, and strong fuchsin.

On the other hand, the action was sharply defined where ammoniacal cupric oxide or water alone was employed, and also where alum dissolved in water was used. In other words, the action is most pronounced when the rays transmitted are those of the blue-violet end of the spectrum, bearing out the results already obtained more generally.

During the progress of the experiments above tabulated, a number of other points of interest were observed. With carbon bisulphide and iodine it frequently happened that no letter was obtained on the plates (Expts. J, 3, III*α*, 7, 9*a*), but occasionally the light action was recorded by the appearance of the letter (Expts. S, III*b*, B_2). The fact is, the solution did transmit a scarcely perceptible amount of violet rays, and since I could not discover any definite relation between the times of exposure and the results, one of two possibilities suggested itself—either differences in the thickness of the glass of the plates, or differences in the degree of clearness of the atmosphere may account for the discrepancies. Probably both causes were effective, for, of course, they both affect these violet rays considerably.

Another phenomenon repeatedly noticed, both in these experiments and in others, was that if the exposure to a very bright sun is continued too long, and especially if the plate is not very accurately at right angles to the direction of the rays, the light may clear the plates entirely, or nearly so. This seems to be due to the reflections of the light from the glass surfaces inside the Petri's dishes; if the light is very intense, or the exposure long, these reflected rays are sufficiently powerful to produce effects similar to those of the direct light.

This seems to me to explain another phenomenon very commonly met with. In many cases of long exposure to clear hot sunshine, the first evidence of the successful light action is not a sharp well-defined letter, but a blurred clear patch, which slowly sharpens up as incubation goes on.

It is evident that in such cases the action of the light has extended beyond the boundaries of the stencil letter, into parts of the film really not exposed to the direct incident rays. I explain this as due to the reflection of some of the rays from the glass surfaces in the interior of the plate.

These reflected rays are not sufficiently intense to complete the bactericidal action, they only inhibit the organism more or less, or at least leave many spores still alive; consequently, while these out-

lying spores germinate more slowly than those further away from the illuminated area—the stencil letter—they *do* at last germinate out, and so the previously blurred letter becomes sharp and clear in outline.

The phenomenon very much resembles the development of the indistinct "ghosts" of letters in cases where the exposure is too short, or the light not sufficiently intense, or wanting in active rays. Such faint letters gradually become obliterated as incubation proceeds, because the spores, still alive but only retarded in development, gradually germinate out to an extent so little differing from the rest that the eye fails to detect any difference.

The retarded development of a few colonies, at a late period of incubation, on the hitherto clear area of the exposed letter, is due to similar causes, but produced in a slightly different way. It is extremely difficult (probably impossible) to thoroughly distribute the spores in the film so that some do not shelter others from the light; consequently, when a clump of spores exists on the exposed area some of the inner spores may so far escape the bactericidal action as to be able to germinate out later, and I have had many experiences of these cases. In fact, the chief point about a good film—*i.e.*, one which develops a sharp letter after ordinary exposure—is that the spores shall be neither too few nor too many, and thoroughly and evenly separated and distributed; and, lastly, that the agar or other medium shall not be too thick, and thus render possible the ordering of long rows of spores one behind another (*i.e.*, in rows parallel to the ray of incident light) which thus shelter one another from the light's action.

These points, and some others, come out still more clearly in the next series of experiments.

§ VII.

The following series of experiments were made behind superposed screens, and it must be borne in mind that the light had to traverse not only a double thickness of solution, but also five thicknesses of glass, before reaching the spores.

On the whole these results may be regarded as simply confirming the previous ones, but I was (and still am) considerably puzzled by the behaviour of the iodine and carbon bisulphide screens. In several cases the plates seem to be destroyed by the light passing through this medium, and for some time I was doubtful whether there might not be a cumulative effect due to the action of the infra-red rays. It seemed extremely probable that these rays—the "dark" heat rays—do help to promote the bactericidal action, and I thought perhaps because they accelerate the chemical changes on which the action depends.

Table G.—Exposures behind Plane-screens Superposed.

Number of plate.	Letter or figure used.	Date made.	Date exposed.	Kind of exposure.	Nature of screen (plane or bottle) if any.	Number of hours sunshine.	Date put into incubator.	Number of days or hours incubated.	Time letters first visible.	Temperature incubated.	Results.	Remarks.
1	Z	Mar. 12	Mar. 12	Direct sun	Plane $CuSO_4Am$ + fuchsin	12—2 P.M.	3 P.M., Mar. 12	$25\frac{1}{2}$ hours	13th	25° C.	Sharp Z out at 4.30 on the 13th. Less sharp next day, growing over.	Extremely good sun, and blue sky. The screens of 1 and 3 transmitted a perceptible amount of violet, whereas that of No. 4 showed no perceptible blue or violet at all. The next experiment shows, however, that the negative result may have been due to under-exposure.
2	E	,,	,,	,,	Plane $CuSO_4Am$ on $CuSO_4Am$	,,	,,	42 ,,	,,	,,	Sharp E at 9 A.M., 14th, but not all killed.	
3	C	,,	,,	,,	Plane fuchsin on fuchsin	,,	,,	,,	,,	,,	C showing at 9 A.M. on 14th, but by no means all killed.	
4	X	,,	,,	Reflected sun	Bottle $CuSO_4$+I and CS_2	12.15—2.25 P.M.	,,	6 days	...	,,	Germinating evenly all over, and apparently no effect.	
15	K	Mar. 18	Mar. 18	Direct sun	Bottle $CuSO_4$+I and CS_2	to 4 P.M.	4.30 P.M., Mar. 18	7 ,,	9 A.M. on 20th	,,	Both letters very faint, and obliterated on 23rd, or nearly so.	Sun hot and bright, blue sky, air cool. Slight haze and glare later.
	Z	,,	,,	,,	Æsculin + quinine	,,	,,	,,				
III	N	Mar. 24	Mar. 25	,,	CS_2+I on alum	11.30 A.M. to 3.30 P.M.	4 P.M., Mar. 25	...	...	...	Negative	Hot sun, but very hazy. Plates ruined by heat.
IV	O	,,	,,	,,	CS_2+I on water	,,	,,					
XII	Z	Mar. 27	Mar. 27	,,	CS_2+I on alum	12 A.M. to 3 P.M.	3 P.M., Mar. 27	3 days	9 A.M. on 28th	25° C.	Extremely faint letters, and only inhibited: obliterated on 29th, except a ghost of K.	Very hot sun, hazy first hour, then brilliant.
XIII	K	,,	,,	,,	CS_2+I on water	,,	,,	,,				

However, on comparing the action of a thick crystal of rock-salt with that of water and alum, I was unable to detect any such marked difference as would seem to follow if that conclusion were correct. As will be seen more clearly later, when I come to discuss the results obtained with the spectrum, the infra-red rays are themselves utterly without perceptible effect.*

§ VIII.

The following series of experiments, designed to estimate the degree of light action on water bacteria, was carried out, under my direction and supervision, by Miss Hayward, of University College, London, and I owe it to her to state that their successful carrying out would have been impossible—on account of the numerous plates to be counted, in short periods, and involving very large numbers—but for her untiring industry and devotion to the work.

Series I.

About 150 c.c. of Thames water, collected at 10 A.M. on August 12, were distributed equally in three Erlenmeyer flasks, properly sterilised, and the flasks labelled A, B, and C; and at 11 A.M. a 1-drop plate was made from each flask. These plates, examined and counted on August 14 at 11 A.M., gave respectively 1560, 1700, and 1080 colonies per c.c.—*i.e.*, an average of 1446 colonies per c.c. developing in two days. On keeping the plates another day, two of them gave 3705 and 1656 respectively, while the third was uncountable and liquefied, the mean being 2680.

We, therefore, assume that the water contained at the outset about 2700 bacteria per c.c., capable of developing in three days.

The flasks were then placed as follows:—A was suspended by the neck so that it could be exposed to what sunshine there was, and at the same time be illuminated from below by the light reflected from a plane silvered mirror.

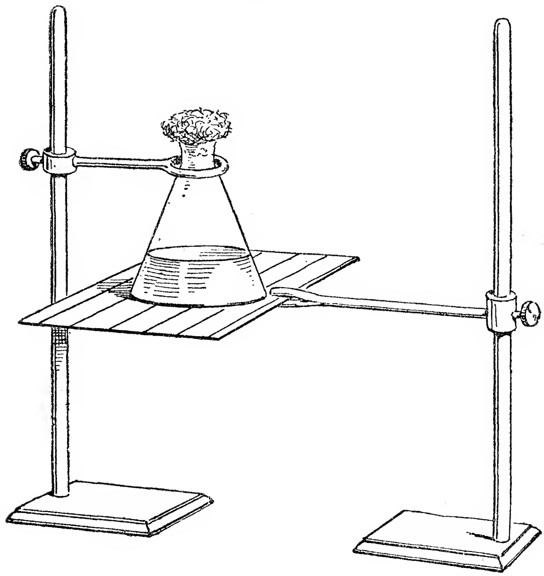
B and C merely stood by the side of the stand supporting A, C being covered with tin-foil and black paper, while B was exposed to the light from above and at the sides.

After standing thus from 12 noon to 4.30 P.M., the weather being very cloudy with only occasional bursts of sunshine, fresh samples were taken from each flask by means of sterilised pipettes, and new plates made to see if any changes had occurred of note.

Taking first the plate from A, after two days' incubation, we found 1599 colonies per c.c.; and after a further twenty-four hours, 2760 per c.c., of which 12 per cent. were liquefying forms. The number of living bacteria capable of developing in two to three days, there-

* Since writing this the experiments with the spectrum have been published separately (See 'Proc. Roy. Soc.,' 1894, vol. 54, p. 472, Abstract).

Fig. 3.



fore, was approximately the same as at the outset, and the dull light seems to have prevented the usual rapid multiplication.

The plate from B, made after exposure, gave us 680 colonies per c.c. after two days' and 2550 colonies per c.c. after three days' incubation, and showed evident signs of liquefaction. Here, therefore, it would seem that the light had exercised an inhibitory action as to numbers.

The plate from C gave 2268 colonies per c.c. after three days' incubation, but it was liquefying still more rapidly.

So far, therefore, with the dull light of a cloudy day, it did not seem as if an exposure of four and a half hours gave results of much significance as to the numbers of bacteria; but it *did* seem as if the plates from the exposed flasks showed less liquefaction.

Meanwhile, the three flasks stood at a temperature of about 16° C. overnight in the laboratory, and were exposed next day from 11.30 A.M. to 4.30 P.M.—again a dull day, and practically no sunshine at all.

Before exposure, however, we took samples as before, and after two days' incubation found that A had about 13,650 per c.c., B had 15,980 per c.c., and C was so badly liquefied that we could place no reliance on the numbers (1584) counted.

These numbers are not very satisfactory taken by themselves, but they showed us that the matter was worth further investigation along similar lines.

The following table H summarises the foregoing facts:—

Table H.

Flask.	Contents.	Exposed or not.	Hours of exposure.	Number of plate.	When made.	When examined.	Number of colonies.	Number of bacteria per c.c.	Remarks.	When water placed in flask.
A.	Thames water	Exposed with mirrors	0	A_1	Aug. 12, 11 A.M.	Aug. 14, 11 A.M. Aug. 15, 12.30 P.M.	40–95	1560–3705		Aug. 12, 11 A.M.
	„	„	$4\frac{1}{2}$	A_2	Aug. 12, 5 P.M.	Aug. 14, noon Aug. 15, 1 P.M.	41–70	1599–2760	Over-night in laboratory, 19 hours at 16° C	„
	„	„	..	A_3	Aug. 13, 11 A.M.	Aug 15, 4 P.M.	350	13,650		„
B	Thames water	Exposed without mirrors	0	B_1	Aug. 12, 11 A.M.	Aug. 14, 11 A.M. Aug. 15, 12.30 P.M.	50 Liquefied	1700 , uncountable		Aug. 12, 11 A.M.
	„	„	$4\frac{1}{2}$	B_2	Aug. 12, 5 P.M.	Aug. 14, noon Aug. 15, 3 P.M.	20–75	680–2550	Over-night in laboratory, 19 hours at 16° C	„
	„	„	..	B_3	Aug. 13, 11 A.M.	Aug. 15, 5 P.M.	470	15,980		„
C	Thames water	Not exposed	0	C_1	Aug. 12, 11 A.M.	Aug. 14, 11 A.M. Aug 15, 12.30 P.M.	30–46	1080–1656		Aug. 12, 11 A.M.
	„	„	0	C_2	Aug. 12, 5 P.M.	Aug. 14, 1 P.M. Aug. 15, 3.30 P.M.	35–63	1260–2268	Over-night in laboratory, 19 hours at 16° C	„
	„	„	0	C_3	Aug. 13, 11.15 A.M.	Aug. 15, 6 P.M.	44*	1584		„

* Actually counted, but badly liquefied, and the number would have been much higher later.

§ IX.

On August 14th, three flasks were prepared and exposed as before; A′ with mirror beneath, B′ with no mirror, and C′ wrapped up. Fairly bright sunshine prevailed during the exposure—from 11.15 to 4.30—an occasional cloud obscuring the sun.

Two samples showed that A′ started with 1755 per c.c. and 1404, the mean being 1578 per c.c. After five hours' exposure, over the mirror, plates were again taken. Three plates yielded 1326, 780, and 858 per c.c., the mean being 988 per c.c., which looks as if a perceptible reduction had occurred.

Two plates from B′ at the start gave 680 and 2176, the mean being 1423 per c.c. After its five hours' exposure, without a mirror, three samples yielded 918, 476, and 476 per c.c., the mean being 623 per c.c., and again suggesting effect of bactericidal rays.

Plates from C′ at the beginning gave 1156 as the number to start with, and after the five hours side by side with the other flasks, but protected from the sunshine by foil and paper, samples gave 3240, 2052, and one uncountable. The mean, = 2646 per c.c., suggesting a perceptible increase.

Here, again, it was evident that liquefaction took place much more rapidly on the plates from the unexposed flask than on those from the flasks exposed to light.

The chief difficulty with these mixed plates is always that caused by the liquefying forms, one of which was especially troublesome, often coming on so rapidly that a plate which looked "safe" at a given time would be ruined three or four hours later.

The foregoing results are summarised in the following Table I.

Without attempting to lay too much stress on the actual numbers in this series, it is pretty evident that if we take the totals or the means of the numbers of bacteria obtained from the water by taking *three* samples from each flask at each period of examination, we get at least some information as to the rate of action of the light on the total organisms.

Put thus, the facts run as follows:—Of the nine samples taken at the start, four were not counted, as they liquefied too rapidly. The average of the other five gave 1434 per c.c.

Flask A′, after five hours' exposure over a mirror, gave 988 per c.c. as the mean of three samples.

Flask B′, after five hours' exposure without a mirror, gave 623 per c.c. as the mean of three plates.

Flask C′, not exposed, but otherwise treated similarly, gave 2646 as the mean of two plates.

It seems impossible to doubt, therefore, that the exposure to light reduced the numbers by nearly one-half. But this proportion becomes

much greater if we note that during the period an enormous multiplication would normally occur.

§ X.

On August 15th two Erlenmeyer flasks were charged as before with Thames water, and labelled A and C. A was exposed over mirrors, and C wrapped up. We introduced the difference here of having an additional mirror *behind* the flask, as well as that below.

The day was bright, with plenty of sunshine all the time, and A was exposed for $5\frac{1}{2}$ hours—from 10.30 a.m. to 4 p.m.—and then samples taken from both.

Meanwhile, the average of seven samples taken at the commencement gave 1644 as the number per 1 c.c. in the Thames water at starting.

Unfortunately, the temperature rose during the next twenty-four hours sufficiently to soften the gelatine of the plates taken after the first $5\frac{1}{2}$ hours, so we could not count these.

On August 16th—another bright, clear day—the flask A was again exposed for six hours, and C (wrapped up) beside it, both flasks having stood all night (nearly 12 hours), at 18° C in a cupboard in the laboratory.

After this second exposure the plates gave—for A about 6000 per c.c., and for C over 174,000 per c.c.; showing that the exposure to the light had kept down the numbers in A, in spite of the interval of twelve hours in a warm, dark cupboard, when, of course, the bacteria not killed off by the first day's exposure multiplied rapidly.

Here, again, I was struck by the diminution of the liquefaction on the plates from the flask A; it did not look like merely fewer liquefying forms, but as if those that were present really liquefied less rapidly and less efficiently than those from the flask not exposed to light.

§ XI.

On August 22nd two Erlenmeyer flasks, marked F 3 and F 4, were charged to a depth of 1 in. with Thames water, properly collected, &c. (see Table J).

Flask F 3 was exposed to the sun with a mirror below; F 4 stood, covered, by its side. The exposure lasted from 11.30 A.M. to 4.30 P.M. (being five hours), but only about one and a half to two hours at most was good sunlight, the day being showery with snatches of blue sky at intervals.

Four samples taken at the beginning of the experiment gave 800, 1408, 748, and 1462 per c.c. as the numbers after two days' incubation. Total of four plates = 4418; average = 1104 colonies per c.c. to start with.

Table I.

Flask.	Contents.	Hour of collection.	Exposed or not.	Hours of exposure.	Number of plate.	When made.	When examined.	Incubation.	Number of colonies.	Number of bacteria per c.c.
A′	Thames water	Aug. 14, 10.30 A.M.	Exposed with mirror	0	A 1′	Aug. 14, 11 A.M.	Aug. 16, 10 A.M.	Hours. 47	45	1755
	”	”	”	5	A 2′	Aug. 14, 5 P.M.	Aug. 16, 11.40 A.M.	42½	34	1326
A′	Thames water	Aug. 14, 10.30 A.M.	Exposed with mirror	0	A 1′	Aug. 14, 11 A.M.	..	..	Liquefied and uncountable	
	”	”	”	5	A 2″	Aug. 14, 5 P.M.	Aug. 16, 2.20 P.M.	46½	20	780
A′	Thames water	Aug. 14, 10.30 A.M.	Exposed with mirror	0	A 1‴	Aug. 14, 11 A.M.	Aug. 16, 10 A.M.	47	36	1404
	”	”	”	5	A 2‴	Aug. 14, 5 P.M.	Aug. 16, 2.20 P.M.	46½	22	858
B′	Thames water	Aug. 14, 10.30 A.M.	Exposed without mirror	0	B 1′	Aug. 14, 11 A.M.	Aug. 16, 11 A.M.	48	20	680
	”	”	”	5	B 2′	Aug. 14, 5 P.M.	Aug. 16, 12 noon	45	27	918

B′	Thames water	Aug. 14, 10.30 A.M.	Exposed without mirror	0	B 1″	Aug. 14, 11 A.M.	Aug. 16, 2 P.M.	Hours. 51	Liquefied and uncountable	
	”	”	”	5	B 2″	Aug. 14, 5 P M.	Aug. 16, 3 P.M.	46	14	476
B′	Thames water	Aug. 14, 10.30 A.M.	Exposed without mirror	0	B 1‴	Aug. 14, 11 A.M.	Aug. 16, 1 P.M.	50	64	2176
	”	”	”	5	B 2‴	Aug. 14, 5 P.M.	Aug. 16, 3 P.M.	46	14	476
C′	Thames water	Aug. 14, 10.30 A.M.	Not	..	C 1′	Aug. 14, 11.30 A.M.	Aug. 16, 11 A.M.	..	Liquefied and uncountable	
	”	”	”	..	C 2′	Aug. 14, 5 P.M.	Aug. 16, 12.30 P.M.	..	90	3240
C′	Thames water	Aug. 14, 10.30 A.M.	..	..	C 1″	Aug. 14, 11.30 A.M.	Aug. 16, 11 A.M.	..	34	1156
	”	”	Not	..	C 2″	Aug. 14, 5 P.M.	Aug. 16, 5.20 P.M.	..	Liquefied and uncountable	
C′	Thames water	Aug. 14, 10.30 A.M.	..	..	C 1″	Aug. 14, 11.30 A.M.	..	..	Liquefied and uncountable	
	”	”	Not	..	C 2‴	Aug. 14, 5 P.M.	Aug. 16, 5.20 P.M.	..	57	2052

After another day's incubation one plate was liquefied; the others gave 832, 1920, and 816. Total of three plates = 3568; average = 1189 colonies per c.c., and this may be taken as the highest number obtainable, the counting having been done and checked very carefully and thoroughly with a good lens.

After the five hours' exposure, new plates were made—two from each of the flasks—and, in order to obviate as far as possible our previous difficulties with the liquefying forms, we diluted each 1 c.c. of the sample water with 9 c.c. of sterile distilled water, carefully prepared in advance.

It is unnecessary to give details as to sterilisation, &c., but the following are the essential points of the plan followed: For *each* sample to be taken two pipettes and two test-tubes are needed. One of the test-tubes of each pair is graduated to hold 9 c.c. of the sterile water; into the other 1 c.c. of the water to be tested is dropped with one pipette, and the 9 c.c. of sterile water are then poured on to this, thus ensuring thorough and rapid mixture. The second pipette is then used to obtain the one or more drops taken to make the gelatine plate.

By this method we found that two plates from F 3 (the exposed flask) gave 2880 and 1280 per c.c. on the 4th day. Total of two plates, 4160; mean, 2080 per c.c., suggesting that the light was not strong enough to prevent the bacteria from multiplying. On standing two days longer these plates gave 3840 and 1600; total, 5440; mean, 2720 colonies per c.c., showing that there were a good many slowly developing germs present, and we were struck with the paucity of liquefying forms.

Two plates from F 4 (the unexposed flask), made and examined at corresponding times, and in the same way, gave 660 and 2310 per c.c. on the fourth day. Total, 2970; mean, 1485 colonies per c.c.; and on the fifth day (we could not go further) 660 and 3630; total, 4290; mean, 2145 colonies per c.c.

So far, therefore, the numbers in the two flasks did not appear to be appreciably affected by the little sunlight that reached the water.

The two flasks were now placed in an ice-safe over night, for exposure next day. They remained on ice about 14 hours.

Next morning, August 23, these flasks were again put out at 9 A.M., and remained till about 4 P.M. (over seven hours); but it rained steadily all the time except the last hour, when the sun shone.

Two plates, made of diluted samples as before, from each flask, were made at noon on this day, with the following results: After two days' incubation the plates from flask F 3 (exposed) showed 39,600 and 37,620 per c.c. Total of two plates, 77,220; mean, 38,610. After a further day's incubation the plates gave 47,190 and 41 250; total, 88,770; mean, 44,385 per c.c.

The two plates from flask F 4 (not exposed), examined at the same time, gave 10,800 and 7,200 per c.c. in two days. Total, 18,000; mean, 9000 per c.c.; and, in three days, they showed 13,200 and an uncountable number, owing to liquefaction.

These results were decidedly mystifying at first, for they showed apparently a stimulating effect of exposure to the light; but on going further into the matter it seems more probable that what really happens is, that (1) the sunlight was not powerful enough in blue-violet rays to produce any appreciable inhibition in the time; and (2) the flask F 4, covered in tin foil, &c., did not become warmed so rapidly as the other, and consequently still showed the retarding action of the icing to which it had been subjected.

That this explanation is right is borne out by the behaviour of the plates taken at 4 P.M.—*i.e.*, after four hours' further exposure of the flasks—for the covered flask, although enormously increased in bacteria, was still behind the exposed one.

After two days' incubation, the two plates from F 3 (exposed) at this period gave 184,800 and 165,000 respectively. Total, 349,800; mean, 179,900 per c.c.; and, after three days, 231,000 and 207,900. Total, 438,900; mean, 219,450 per c.c.

Whereas the two plates from the non-exposed flask gave respectively 19,500 and 12,000 per c.c.; total, 31,500; mean, 15,750 per c.c., after two days' incubation, and were not counted further.

Considering the enormous expenditure of time and trouble involved in making and counting these plates, we were somewhat discouraged by these negative results, which are summarised in the accompanying Table J (p. 346, &c.).

§ XII.

On August 24th two flasks of Thames water labelled F 5 and F 6 were exposed exactly as the last, but the weather was fine and we had much bright sun and blue sky, with rapidly moving white clouds.

The first exposure was from 9.30 A.M. to 4 P.M., and the arrangement as before. The temperature, as indicated by thermometers in control flasks, rose occasionally over 35° C, but was usually not above 30° C, and somewhat higher in the covered flask than in the uncovered one.

Four plates of the water with which the flasks were charged were made at the time of starting the experiment, and were incubated as long as possible. In four days they gave us 1980, 1650, 1320, and 2970 colonies per c.c. Total of the four samples, 7920; average, 1980 colonies per c.c.

After six and a half hours' exposure, of which about four hours was brilliant sunshine as far as we could estimate, two plates from each

Table J.

Flask.	Contents.	When filled.	Exposed or not.	Time of exposure.	Number of hours exposed.	Number of hours of sun.	Other treatment.	Number of plate.	When made.
F 3	Thames water (collected Aug. 22, 10 A.M.) ½ in. deep	Aug. 22, 11.30 A.M.	Not	..	0	0	..	P 17	Aug. 22, 11.30 A.M.
,,	,,	,,	,,	..	0	0	..	P 18	,,
F 4	,,	,,	,,	..	0	0	..	P 19	,,
,,	,,	,,	,,	..	0	0	..	P 20	,,
F 3	,,	,,	Exposed mirror below	Aug. 22, 11.30 A.M.—4.30 P.M.	5	$1\frac{1}{2}$—2	..	P 23	Aug. 22, 5 P.M.
,,	,,	,,	,,	,,	5	$1\frac{1}{2}$—2	..	P 24	,,
F 4	,,	,,	Not	..	0	0	..	P 25	,,
,,	,,	,,	,,	..	0	0	..	P 26	,,
F 3	,,	,,	Exposed mirror below	Aug. 22, 11.30 A.M.—4.30 P.M. Aug. 23 9 A.M.—12 noon & 12—4.30	$7\frac{1}{2}$	$1\frac{1}{2}$—2	Flask left at laboratory, temperature 16—18° over-night	P 27	Aug. 23, 12 noon.
,,	,,	,,	,,	,,	$7\frac{1}{2}$	$1\frac{1}{2}$—2	,,	P 28	,,

Table J.

When examined.	Hours of incubation.	Temperature of incubation.	Number of colonies on plate.	Number of bacteria per c.c.	Remarks.	Weather.
(1) Aug. 24, 1 P.M.	49½	16—18°	25	800	7 small liquefying colonies.	
(2) Aug. 25, 3 P.M.	75½	,,	26	832	10 liquefying colonies.	
(1) Aug. 24, 2.30 P.M.	51	,,	44	1,408	6 liquefying.	
(2) Aug. 25, 3 P.M.	75½	,,	60	1,920	14 liquefying.	
(1) Aug. 24, 3 P.M.	51½	,,	22	748	6 liquefying.	
(2) Aug. 25, 3 P.M.	75½	,,	24	816	9 liquefying.	
(1) Aug. 24, 3 P.M.	51½	,,	43	1,462	11 liquefying.	
(2) Aug. 25, 3 P.M.	75½	,,	liquefied and therefore uncountable			
(1) Aug. 26, 9 A.M.	88	,,	9	2,880	1 c.c. diluted to 10 with sterile distilled water, and 1 drop of the mixture taken. None liquefying. 1 mould present additionally	Heavy clouds and showers, with blue sky and sunshine between.
(2) Aug. 27, 12.30 P.M.	115½	,,	12	3,840	Diluted. 1 mould	Ditto.
(3) Aug. 28, 12.30 P.M.	139½	,,	..	..	2 small liquefying. 2 moulds	Ditto.
(1) Aug. 26, 9 A.M.	88	,,	4	1,280	Diluted. None liquefying. 1 mould	Ditto.
(2) Aug. 27, 12.30 P.M.	115½	,,	5	1,600	Ditto	Ditto.
(3) Aug. 28, 12.30 P.M.	139½	,,	..	..	1 slow liquefier appeared.	Ditto.
(1) Aug. 26, 9.30 A.M.	88½	,,	2	660	Diluted. No liquefying Plate on ice till next morning 9 A.M.	
(2) Aug. 27, 1 P.M.	115½	,,	2	660	2 moulds appeared.	
(1) Aug. 26, 9.30 A.M.	88½	,,	7	2,310	No liquefying.	
(2) Aug. 27, 1 P.M.	115½	,,	11	3,630	No liquefying.	
(1) Aug. 26, 10 A.M.	70	,,	120	39,600	Diluted. 4 small liquefiers	Steady rain all the morning; an hour's sun 3—4 P.M., but feeble.
(2) Aug. 27, 4 P.M.	100	,,	143	47,190	9 liquefiers. 1 mould appeared	
(3) Aug. 28, 12.30 P.M.	120½	,,	..	..	11 good sized liquefiers, and many small ones	
(1) Aug. 26, 10 A.M.	70	,,	114	37,620	Diluted. 5 liquefying, all small	Ditto.
(2) Aug. 27, 4.30 P.M.	100½	,,	125	41,250	Diluted. 16 liquefying	Ditto.

Table J—

Flask.	Contents.	When filled.	Exposed or not.	Time of exposure.	Number of hours exposed.	Number of hours of sun.	Other treatment.	Number of plate.	When made.
F 4	Thames water (collected Aug. 22, 10 A.M.) ½ in. deep	Aug. 22, 11.30 A.M.	Not	..	0	0	Flask left at laboratory, temperature 16—18° over-night.	P 29	Aug. 23, 12.30 P.M.
"	"	"	"	..	0	0	"	P 30	"
F 3	"	"	Exposed	Aug. 22, 11.30—4.30 Aug. 23, 9—12 12—4.30	12½	2½—3	"	P 31	Aug. 23, 4.30 P.M.
"	"	"	"	"	12½	2½—3	"	P 32	"
F 4	"	"	Not	..	0	0	"	P 33	Aug. 23, 5 P.M.
"	"	"	"	..	0	0	"	P 34	"

flask were made, and incubated also as carefully and long as possible, to get the maximum numbers.

The two plates from the exposed flask F 5 gave 160 and 320 after four days' incubation. Total, 480; mean, 240 per c.c.; whereas those from the unexposed flask gave 3400 and 5916 respectively in the same period. Total, 9316; mean, 4658 colonies per c.c.

These numbers show very distinctly the effect of the sunshine, and are borne out clearly by what follows.

After the exposure on August 24th, and after the samples for plates had been taken, both flasks were placed on ice over-night, and remained on ice about fourteen hours.

On the 25th, at 9.30 A.M., the exposure was repeated, the flasks being first examined as to the effects of their sojourn in the ice-box. The numbers were found to have remained remarkably constant, being 210 for the exposed and 3136 for the covered flask.

continued.

When examined.	Hours of incubation.	Temperature of incubation.	Number of colonies on plate.	Number of bacteria per c.c.	Remarks.	Weather.
(1) Aug. 26, 10 A.M.	69½	16—18°	32	10,800	Diluted. 8 liquefying.	
(2) Aug. 27, 4.30 P.M.	100	”	44	13,200	” ”	
(1) Aug. 26, 11 A.M.	68½	”	24	7,200	” 3 liquefying.	
(2) Aug. 27, 4 P.M.	99½	”	liquefied and therefore uncountable			
(1) Aug. 26, 10.30 A.M.	66	”	560	184,800	Diluted. 15 liquefying	Steady rain all the morning; an hour's sun 3—4 P.M., but feeble.
(2) Aug. 27, 5 P.M.	96½	”	700	231,000	Diluted. About 55 liquefying	Ditto.
(1) Aug. 26, 11 A.M.	66½	”	500	165,000	7 large and many small liquefying.	Ditto.
(2) Aug. 27, 5 P.M.	96½	”	630	207,900	Diluted	
Aug. 28, 11.30 A.M.	66½	”	65	19,500	Diluted. 6 liquefying. 1 mould.	
”	”	”	40	12,000	Diluted. 1 liquefying.	

After this day's exposure, which lasted six hours, about half of which was more or less cloudy at intervals and the rest bright sunshine, the plates made again showed great differences, clearly pointing to the inhibitory action of the sunlight, for the sample of exposed water gave 1584 as against 29,760 per c.c. in the unexposed flask.

In other words, although flask F 5 had stood fourteen hours in the dark at a temperature not too low for increase, its exposure of about 12½ hours to the light had resulted in the reduction of its bacteria to a number below that it started with; the unexposed flask meanwhile had its bacteria multiplying normally at the rapid rates usual for these waters. Had the sunshine been more intense during this second day, it is by no means improbable that the water could have been completely sterilised by exposure.

The results discussed above are put into a tabular form in the following Table K:—

Table

Flask.	Contents.	When filled.	Exposed or not.	Time of exposure.	Hours of exposure.	Hours of sun.	Other treatment.	Plate.	When made.
F 6	Thames water 1 in. deep. Collected Aug. 24, 9.10 A.M.	Aug. 24, 9.30 A.M.	Not	..	0	0	..	P 37	Aug. 24, 10 A.M.
,,	,,	,,	,,	..	0	0	..	P 38	,,
,,	,,	,,	,,	..	0	0	..	P 39	,,
,,	,,	,,	,,	..	0	0	..	P 40	,,
F 5	,,	,,	Exposed with mirror	Aug. 24, 9.30 A.M.—4 P.M.	$6\frac{1}{2}$	4	..	P 39*a*	Aug. 24, 5 P.M.
,,	,,	,,	,,	,,	$6\frac{1}{2}$	4	..	P 40*a*	,,
F 6	,,	,,	Not	..	0	0	..	P 41	,,
,,	,,	,,	,,	..	0	0	..	P 42	,,
F 5	,,	,,	Exposed with mirror	Aug. 24, 9.30 A.M.—4 P.M.	$6\frac{1}{2}$	4	After having been placed on ice overnight from 5.30 P.M.—9.30 A.M.	P 43	Aug. 25, 9.30 A.M.
F 6	,,	,,	Not	..	0	0	,,	P 44	Aug. 25, 10 A.M.
F 5	,,	,,	Exposed with mirror below	Aug. 24, 9.30 A.M.—4 P.M. Aug. 25, 9.30 A.M.—10.30 P.M.	$12\frac{1}{2}$	$7\frac{1}{2}$	..	P 49	Aug. 25, 3.30 P.M.
F 6	,,	,,	Not	..	0	0	..	P 50	,,

§ XIII.

On August 25 two flasks labelled F 7 and F 8 were exposed as the last, from 9.30 A.M. to 4 P.M., weather mixed—clouds and bright sunny intervals.

Four samples taken at the beginning gave 1666, 1292, 1394, and 1768 colonies per c.c.; total, 6120; average, 1530 germs per c.c. to start with.

K.

When examined.	Hours of incubation.	Temperature of incubation.	Number of colonies on plate.	Number of bacteria per c.c.	Remarks.	Weather.
Aug. 28, 12 noon	98	16—18°	6	1,980	Diluted. 3 moulds also present.	
,,	,,	,,	5	1,650	Diluted. 2 moulds also present.	
,,	,,	,,	4	1,320	Diluted. 1 grey liquefier. 1 mould also.	
,,	,,	,,	9	2,970	Diluted. 2 liquefying.	
Aug. 28, 12.30 P.M.	91½	,,	5	160	No dilution. 5 moulds also present	Rapidly moving clouds, blue sky, and bright sun.
,,	,,	,,	10	320	3 moulds also present	,,
(1) Aug. 27, 5 P.M.	72	,,	90	3,060	11 liquefying.	
(2) Aug. 28, 12.30 P.M.	91½	,,	100	3,400	Very much liquefied.	
,,	91½	,,	174	5,916	9 liquefying. 1 small mould also.	
Aug. 28, 12.30 P.M.	75	,,	7	210	None liquefying.	
Aug. 28, 1 P.M.	75	,,	98	3,136	6 liquefying.	
Aug. 28, 4.30 P.M.	73	,,	48	1,584	3 liquefying and 1 mould also	Clouds and bright sunshine.
,,	73	,,	930	29,760	About 27 liquefying.	

After the six and a half hours' exposure two plates were made from each flask. The two from F 7 (exposed) gave 93 and 31 per c.c.; total, 124; mean, 62 colonies per c.c.; while those from F 8 (covered) in the same period of incubation, viz., four days, gave 2190 and 2130; total, 4320; mean, 2160 per c.c.

Here again, therefore, the direct sunshine has a decided bactericidal effect, as is clear from details in the following Table L:—

Table

Flask.	Contents.	When filled.	Exposed or not.	Time of exposure.	Hours of exposure.	Hours of sun.	Plate.	When made.
F 8	Thames water 1 in. deep. Collected Aug. 25, 9.10 A.M.	Aug. 25, 9.30 A.M.	Not	..	0	0	P 45	Aug. 25, 10.30 A.M.
"	"	"	"	..	0	0	P 46	"
"	"	"	"	..	0	0	P 47	"
"	"	"	"	..	0	0	P 48	"
F 7	"	"	Exposed mirror below and behind	Aug. 25, 9.30 A.M. — 4 P.M.	$6\frac{1}{2}$	4	P 51	Aug. 25,4 P.M.
"	"	"	"	"	$6\frac{1}{2}$	4	P 52a	"
F 8	"	"	Not	..	0	0	P 53a	"
"	"	"	"	..	0	0	P 54a	"

§ XIV.

On August 28 another pair of flasks (F 9 and F 10) were exposed as before, 10.30 A.M. till 6.80 P.M., and left out all night; exposed next day from 10 A.M. to 4 P.M. The sky was cloudy on both days, but there was a good deal of sunshine also, particularly on August 29. We estimated the actual exposure to daylight as 18 hours, and about 8 hours' good sun altogether.

In order to extend our numbers whence we drew the average of bacteria present at the commencement, *twelve* samples were taken for plates to begin with, and we diluted to 1 in 10 as before.

After three days' incubation we found four of the plates too far liquefied to count, but the numbers would probably not be inconsistent with the following :—

The eight plates gave a total of 10,890 colonies, and an average of 1361 colonies per c.c. in the water at the commencement of the experiment.

After the exposure to light and darkness from 10.30 A.M. August 28 to 4 P.M. August 29, we found about 300 per c.c. in the flask ex-

L.

When examined.	Hours of incubation.	Temperature of incubation.	Number of colonies on plate.	Number of bacteria per c.c.	Remarks.	Weather.
Aug. 28, 3.30 P.M.	77	..	49	1666	6 liquefying; also 1 mould appeared.	
Aug. 30, 12 noon	121½	..	100	3400		
Aug. 28, 4 P.M.	77½	..	38	1292	6 liquefying.	
Aug. 30, 12 noon	122	..	64	2716		
Aug. 28, 4 P.M.	77½	..	41	1394	5 liquefying; also 1 mould.	
Aug. 30, 11.30 A.M.	121½	..	79	2686		
Aug. 28, 4 P.M.	77½	..	52	1768	9 liquefying.	
Aug. 30, 10.30	119½	..	136	4624		
Aug. 29, 11.30 A.M.	91½	..	3	93	1 mould also..........	Clouds and bright sunshine.
"	91½	..	1	31		
(1) Aug. 28, 4.30 P.M.	72½	..	58	1740	5 liquefying.	
(2) Aug. 29, 11 A.M.	91	..	73	2190	6 liquefying; also 1 mould.	
Aug. 29, 11.30 A.M.	91½	..	71	2130	5 liquefying.	

posed to the light, and numbers too high to count in that covered with foil and paper; moreover, the latter plates were so badly liquefying that their marked contrast to the former could not escape observation.

It was clear that exposure to sunlight affects the liquefying powers of the forms in the Thames water, and this apart either from the difference in numbers on the plates or because it eliminates these forms more rapidly.

§ XV.

On August 29 two flasks were prepared as before. F 11 was exposed to sun with mirrors, &c., and F 12 put by its side covered with foil and black paper.

The first exposure was from 9.30 A.M. to 12.30 A.M.—three hours' good sunshine, though with occasional clouds.

The water to start with was estimated to contain about 1200 germs per c.c.

After three hours' exposure F 11 gave, as the result of two plates,

99 and 132; total = 231; mean = 115 per c.c. After the same time F 12 (unexposed) gave 1600 and 1920; total, 3520; mean, 1760.

The flasks were meanwhile put back and exposed yet another $4\frac{1}{2}$ hours to the afternoon sunshine—*i.e.*, from 12.30 to 5 P.M.—of which about $3\frac{1}{2}$ hours counted as bright sunshine.

Two plates from F 11 made at 5.30 gave 248 and 279; total, 527; mean, 268 colonies as the number per c.c.; while two plates made at the same time from the unexposed flask (F 12) were so badly liquefied that, although we estimated 3300 per c.c. from one of them, we regard the numbers as really higher.

These flasks stood in the laboratory over-night at a temperature of 18° C., and were then exposed next day from 11.30 A.M. to 4.30 P.M., about one and a half or two hours of the five being sunny. Then, at 5 P.M., fresh plates were prepared.

Two plates from the exposed flask gave 640 and 3200 per c.c. respectively; total, 3840; mean, 1920 per c.c. The numbers are not very good, as there is such a great difference between the two plates.

Two plates from the unexposed flask gave 12,800 and 16,000 per c.c.; total, 28,800; mean, 14,400 per c.c., again bearing out the conclusion that the sun has powerful action on the exposed water.

§ XVI.

To my mind one of the most important discoveries elicited from these plate cultures of Thames water was the obvious reduction of liquefaction on the plates made from water exposed to light, and so struck was I with the differences between these plates and those made with the water not exposed that I made an independent investigation into the matter by selecting a set of the most pronounced liquefying forms from the water and examining their behaviour when exposed to light side by side with that of non-exposed samples.

I started with the commonest and most pronounced liquefying form in the Thames water at the time. I refer to it throughout as Colony β in my notes, and write it shortly β. I had noticed the following facts concerning it during our experiments on the action of light on the Thames water as collected—it should be borne in mind that I had already studied its characters and knew the form pretty well.

In the first place the plates as a whole liquefied very much more slowly and less completely than those made from unexposed water. Secondly, although it seemed at times as if this was because the form β had been eliminated from the water, I suspected that a certain other form, which liquefied less rapidly and developed much more slowly altogether, was really the above-mentioned form β with feebler characteristics.

On September 3 a small loopful of a gelatine culture of the bacillus β was carefully shaken in about 50 c.c. of sterile water, and distributed equally in two Erlenmeyer flasks labelled β (1) and β (2).

Flask β (1) was wrapped in foil and black paper; β (2) was exposed over mirror from 9.30 A.M. to 5 P.M., and about five hours of these seven and a half were good strong sunshine. Thermometers in control flasks went up to 34—35° C. as the highest temperature registered in the afternoon.

At 9.30 a plate was prepared from each flask, and gave something like 3,000,000 per c.c. as the average numbers to start with.

After exposure, two plates were prepared from each. Of the two plates from the exposed flask, one plate—a 1-drop plate of a 1/10th dilution—yielded no colonies at all; the other gave 21,941 per c.c., pointing to a profound light-action.

Of the two plates from the darkened flask, one gave 5,400,000, and the other 5,000,000 as the nearest estimate per c.c.

The two flasks meanwhile stood over-night in the laboratory at 18° C. for about fourteen hours, and at 7 A.M. next day (September 4) I made two plates from the exposed flask and then again put them out as before.

These two plates gave 42,000 and 59,220 respectively; total, 101,220; mean, 50,610 bacteria per c.c., a prefectly natural rise in the numbers having occurred during the night.

No plates were taken from the other flask, as I had no particular need for the numbers—known to be very high—and wished to reserve the counting for other plates.

After exposure to the bright sunshine of September 4, from 8 to 4.30, say eight hours' sunshine on the exposed flask, two new plates were made from each flask.

The two plates from exposed flask gave 3050 and 4200; total, 7250; mean, 3625 bacteria per c.c., again showing a marked reduction in the sunlight, and the plates were singularly free of liquefying centres, but showed many colonies of the kind I had previously suspected as being the representatives of β.

Of the two plates from the unexposed flask, although made from one drop each of a 1/20th dilution, the numbers were again so large and the liquefaction so rapid that no reliance can be placed on them, except that they prove that no essential diminution was to be traced to the action of the water or temperature in the absence of light, but only the natural fall in numbers always found when water stands for some time. The numbers actually calculated were 660,000 and 3,300,000; total, 3,960,000; mean, 1,980,000 per c.c.

The flasks stood in the laboratory at 18° C. through the night, and I repeated the exposure on September 5, putting the uncovered flask into the bright sunlight of that day *without* a mirror. It received a

Table M.—Experiments on Insolation

Flask.	When filled.	Exposed or not.	Time of exposure.	Hours of exposure.	Hours of sun.	Other treatment.	Plate.
β (1)	Sept. 3, at 9 A.M.	Not	..	..	..	..	P. β (1)
β (2)	,,	..	..	..	..	..	P. β (2)
β (1)	,,	..	..	..	..	..	Pa. β (1)
β (1)	,,	..	..	..	..	..	Pb. β (1)
β (2)	,,	Exposed	9.30 to 5 on Sep. 3.	$7\frac{1}{2}$	5	Stood above and in front of mirror	P^{i} β (2)
β (2)	,,	,,	,,	,,	,,	,,	P^{ii} β (2)
β (2)	,,	,,	,,	,,	,,	Ditto. Then = 14 hours in dark at 18°C.	P^{iii} β (2)
β (2)	,,	,,	,,	,,	,,	,,	P^{iv} β (2)
β (2)	,,	,,	9.30 to 5 on Sep. 3 and 8 to 4.30 Sep. 4	16	13	,,	P^{v} β (2)
β (2)	,,	,,	,,	,,	,,	,,	P^{vi} β (2)
β (1)	,,	Not	..	..	..	In dark whole time = 40 hours	Pc β (1
β (1)	,,	,,	..	..	..		Pd β (1)
β (1)	,,	,,	..	..	..	In dark 64 hours	Pe β (1)
β (2)	,,	Exposed	9.30 to 5 on Sep. 3, 8 to 4.30 Sep. 4, 10 to 5 Sep. 5.	23	19	In dark at 18° each night	P^{vii} β (2)

good six hours' sun direct, and the temperature rose as high as 37° C. at one time, but was for the most part at 30—32° C. All the other conditions were as before.

After exposure, a plate from the insolated flask gave 665 per c.c. One from the covered flask gave 2,600,000 as the nearest estimate I could form.

It is obvious, therefore, that the bacterium referred to as Colony β is very sensitive to the solar action, and the results obtained with the above pure cultures are summarised in Table M.

§ XVII.

The results with a second badly liquefying form, which I call

of Bacillus β in Sterile Water.

When made.	When examined.	Time of incubation.	Temperature of incubation.	Dilution or not.	Quantity used.	No. of colonies counted on plate.	Number of bacteria per c.c.
		days.	C°.		c.c.		
9.30 A.M., Sep. 3	Sep. 8	5	16—18	$\frac{1}{20}$	$\frac{1}{30}$	About 5,000	3,000,000
..	..	..	..	$\frac{1}{20}$	$\frac{1}{36}$	5,844 actually counted	3,506,400
5 P.M., Sep. 3	..	..	..	$\frac{1}{10}$	$\frac{1}{36}$	30 squares averaged 500 per sq. = 15,000	5,400,000
..	..	..	..	$\frac{1}{20}$	$\frac{1}{35}$	Very similar numbers	5,000,000
..	..	..	..	$\frac{1}{10}$	$\frac{1}{36}$	0	
..	..	..	..	0	$\frac{1}{37}$	593	21,941
7 A.M., Sep. 4	Sep. 15	11	..	$\frac{1}{20}$	$\frac{1}{21}$	100	42,000
..	..	..	..	$\frac{1}{20}$	$\frac{1}{21}$	141	59,220
4.30 P.M., Sep. 4	..	..	..	0	$\frac{1}{25}$	122	3,050
..	..	..	..	$\frac{1}{10}$	$\frac{1}{35}$	12	4,200
..	..	..	..	$\frac{1}{20}$	$\frac{1}{33}$	About 1,000	660,000
..	..	..	..	$\frac{1}{20}$	$\frac{1}{33}$	About 5,000	3,300,000
5 P.M., Sep. 5	..	10	..	$\frac{1}{20}$	$\frac{1}{26}$	About 5,000	2,600,000
..	..	..	..	$\frac{1}{19}$	$\frac{1}{35}$	1	665

Bacillus η, are not contradictory of the foregoing—indeed they support them so far as they go—but are less conclusive in detail.

On September 5 two Erlenmeyer flasks charged with sterile distilled water, to which a loopful of the bacillus in question was added, were placed out in the usual way. Flask labelled η (1) was covered; flask η (2) was exposed over and in front of plane mirrors.

A plate from each flask at the beginning gave respectively 1,470,144 and 1,699,360; total, 3,169,504; mean, 1,584,752, as the number of bacteria per c.c. to start with.

The flasks were out from 10 A.M. to 4.30 P.M., the day being beautifully bright with a hot sun and blue sky. The temperature in the covered flask rose to 33° C. in the afternoon, that in the exposed

one to about 34° C., as shown by controls with thermometers in the water.

Two samples of the water of the unexposed flask, taken at 4.30 P.M. on September 5, gave 1,575,860 and 1,285,388 as the numbers per c.c.; total, 2,861,248; mean, 1,430,624 per c.c., suggesting that the sojourn in distilled water at that temperature, even in the dark, causes the death of large numbers of this bacillus.

Of two samples taken at 4.30 from the flask η (2), which had been exposed for six and a half hours, neither plate gave any sign of life after ten days' incubation, whence we may assume that neither sample contained a living germ.

These two flasks were put in a cool cupboard over-night—temperature = 15° C.—and again put out on the 6th September from 9.30 to 4.30, so that the exposed flask—η (2)—received another good six hours of bright sun, for the day was brilliantly fine again.

After the exposure, a plate was made from each flask. That from η (2), the one exposed to the sun, gave no signs of life though incubated for ten days; the other showed 560,000 per c.c.

It seems probable, therefore, that in the case of Bacillus η the immersion in sterile water at 33—34° C., even in the dark, is more or less fatal, for we see the bacteria are reduced from over a million and a half per c.c. to nearly half a million per c.c. At the same time it seems pretty clear that when exposed to light at the same time the mortality of the bacilli is much greater. The inference appears fair, but there is naturally some dissatisfaction to be felt with these negative results.

The following table N summarises these facts:—

Table N.—Experiments on Insolation

Flask.	When filled.	Exposed or not.	Time of exposure.	Hours of exposure.	Hours of sun.	Other treatment.	Plate.
η (1)	Sept. 5, 10 A.M.	Not	..	..	..	..	η (1)
η (2)	,,	,,	..	..	..	..	η (2)
η (1)	,,	,,	..	..	..	Covered in the open all day	$\alpha\ \eta$ (1)
η (1)	,,	,,	..	..	..	,,	$\beta\ \eta$ (1)
η (2)	,,	Exposed	10 to 4.30 on Sept. 5	6½	6	Over and in front of plane mirror	I η (2)
η (2)	,,	,,	,,	..	,,	,,	II η (2)
η (1)	,,	Not	..	..	..	Stood over-night at 15°	$c\ \eta$ (1)
η (2)	,,	Exposed	10 to 4.30 Sept. 5, and 9.30 to 4.30 Sept. 6	7	6	,,	III η (2)

* There were 32 squares, averaging about 700

§ XVIII.

On August 20, two Erlenmeyer flasks, labelled F_1 and F_2, were charged to a depth of about an inch with sterile-distilled water with which a loopful of spores of *B. anthracis* had been thoroughly shaken up. Flask F_1 was exposed with a mirror below and one behind; F_2 was wrapped in foil and black paper.

The exposure was from 11.30 A.M. to 5 P.M., but it was a windy and cloudy day, with a good deal of rain. Just before exposure, two plates were made to determine the number of spores introduced per c.c. in the flask F_1. One plate gave 1,950,000, and the other 2,445,000; total, 4,395,000; mean, 2,197,500 per c.c.

Two plates from F_2 gave respectively 2,052,000 and 2,280,000; total, 4,332,000; mean, 2,166,000.

Or, if we take the average of the four plates, we get total = 8,727,000; average of the four = 2,181,750; and it will be noticed how well the four plates agreed.

At 5.30 P.M. two plates were made from the covered flask, and gave per c.c. 1,700,000 and 340,000 respectively. It was noted, however, that the second plate had been badly levelled, and the colonies were heaped up to one side, and could not be properly estimated. Taking the numbers as they stand, we get total = 2,040,000; mean = 1,020,000 per c.c., indicating some reduction, but still enormously high numbers present.

Two plates from the exposed flask F_2, after the five and a half hours' insolation, gave 595,000 and 1,295,000; total, 1,890,000; mean, 945,000.

of Bacillus η in Sterile Water.

When made.	When examined.	Time of incubation.	Temperature of incubation.	Dilution or not.	Quantity used.	No. of colonies on plate.	No of bacteria per c.c.
		Days.			c.c.		
Sept. 5, 10 A.M.	Sept. 8	3	15—18°	$\frac{1}{19}$	$\frac{1}{26}$	2976	1,470,144
,,	,,	,,	,,	,,	,,	3440	1,699,360
Sept. 5, 4.30	,,	,,	,,	,,	,,	3190	1,575,860
,,	,,	,,	,,	,,	,,	2602	1,285,388
,,	Sept. 15	10	,,	,,	$\frac{1}{28}$	0	0
,,	,,	,,	,,	,,	,,	0	0
Sept. 6, 4.30	Sept. 9	3	,,	0	$\frac{1}{25}$	22,400*	560,000
,,	Sept. 16	9	,,	0	$\frac{1}{28}$	0	0

per square as near as could be counted.

This seemed to show that the exposure to what was, after all, only diffused light, had very little effect in five and a half hours.

Both flasks were taken in at 5.30, and put on ice for the night at 6 P.M., and remained on ice till 11 A.M. on the 21st, *i.e.*, seventeen hours in dark and on ice. They were then put out again from 11.15 A.M. to 4 P.M., the weather being much brighter, though plenty of white cumulus clouds kept sweeping over the sun.

Before putting out, two plates were made from each flask at 11.30 A.M. on the 21st. Those from F_1 (unexposed) gave 1,149,000 and 646,000; total, 1,795,000; mean, 897,500; numbers very similar to those of the previous day, and indicating that no essential changes had occurred on the ice—possibly a few had succumbed to the rapid cooling.

The two plates from the exposed flask F_2 gave 42,000 and 1,200,000—the last number being too high, as there were numerous invading forms on the plate rendering it difficult to count. Taking the figures as they stand we have, total, 1,242,000; mean, 621,000, which is a reduction on last night's figures.

After exposure on the 21st, two plates were again made from each flask, with the following results.

Of the two plates from the exposed flask F_1, one gave 122,500, and the other 50,750 as the maximum numbers per c.c. Total, 173,250; mean, 86,625 per c.c.

While the unexposed flask gave 1,920,000 and 640. The last low number was obviously due to some blunder; but, even if we take it to reduce the average, we get total, 1,920,640; mean, 960,320 per c.c.

After exposure, the flasks were again put on ice at 9 P.M., and remained there till 12 noon next day, *i.e.*, August 22; they had been at 16° C. in the interval from 4.30 P.M. to 9 P.M.

On the 22nd a plate was taken from each flask at 2.30 to 3 P.M., and the F_1 gave no anthrax colonies at all, though nursed for nearly a week. The other flask gave 990 colonies, which comes to 297,000 per c.c.

On the 23rd, after another seven and a half hours' exposure, plates were again made, one from each, and gave the following numbers—the exposed flask 2 colonies, which = 720 per c.c., and shows that all spores were not yet killed, and the unexposed one 20 colonies, which = 6800 per c.c.

Unfortunately we were compelled to abandon these flasks now; the mere labour of counting within the necessary periods the numerous plates we were making made it imperative that this series should be discontinued. I am now particularly sorry this was so, because it would have been interesting to find if, and when, the light absolutely cleared the water of spores. However, we could not foresee what the tabular *résumé* brings out so clearly. (Table O.)

A point of great importance arises here—not for the first time, but very vividly. That is the gradual, and much slower, but, nevertheless, determined reduction of the spores, even in the dark flask. I am convinced that the principal factor in this is the changes in temperature undergone by the water, which was warmed up to 30° C., or thereabouts, during the day, and cooled to 4° or 5° C., or even lower, during its stay on ice.

§ XIX.

The following experiment (Table P) gives an excellent example of how much can be done by the clear sun of a hot summer day in clearing the water of living spores of anthrax.

A quantity of anthrax spores were carefully rubbed up in about 50 c.c. of sterile-distilled water, on August 16, and the infected water distributed into two Erlenmeyer flasks, marked A*x* and B*x*.

A*x* was exposed to the sun, with a mirror below and behind, from 10 A.M. to 4 P.M., and the sun during the whole period was brilliant. B*x* was placed beside A*x*, but carefully shut in an opaque wooden box.

A sample plate was taken from each flask before exposure, and, after twenty-five and a half hours' incubation at 22—25° C., gave the following numbers. Plate from A*x* = 23,000 colonies = 897,000 per c.c., the plate beginning to liquefy. Plate from B*x*, after the same incubation, was already in an advanced stage of liquefaction, but we satisfied ourselves of at least 5000 visible colonies = 170,000 per c.c. Total of the two, 1,067,000; mean, 533,500, as the minimum number per c.c.

At 4.15 to 4.30 P.M., after six hours' bright insolation of the exposed plate, two sample plates were taken from each flask.

Those from A*x* (exposed) gave 117 and 40 colonies respectively as the maximum numbers we could discover after 72½ hours' incubation, beyond which we could not carry the process. The counting was done twice every day, and every colony actually marked. These numbers give us 4563 and 1360 per c.c. as the maximum; total = 5923; mean = 2961 per c.c.

On the two plates from B*x* (not exposed) we found *at least* 5900 and 6600 respectively, after repeated countings of all the squares. Moreover, these numbers were obtained in forty-eight and a half hours, the liquefaction being so pronounced later that we could not count further. We thus get a *minimum* of 200,600 and 228,400 per c.c.; total, 429,000; mean, 219,500 per c.c.

Even admitting—as of course we do—that these numbers can only be approximations, it is at least clear that the bactericidal power of the sun's rays, even on the spores in sterile water, is far more

Table O.—Insolation of Anthrax

Flask.	Contents.	When filled.	Exposed or not.	Time of exposure.	Number of hours exposed.	Number of plate.	When made.	When examined.
F 1	Anthrax spores in sterile distilled water ½ in. deep	Aug. 20, 11 A.M.	Not	..	0	P 1	Aug. 20, 11 A.M.	Aug. 24, 9 A.M.
F 1	”	”	”	..	0	P 2	”	Aug. 23, 10 A.M.
F 2	”	”	”	..	0	P 3	Aug. 20, 11.30 A.M.	Aug. 23, 11 A.M.
F 2	”	”	”	..	0	P 4	”	”
F 1	”	”	Exposed	Aug. 20, 11.30 A.M. —5 P.M.	5½	P 5	Aug. 20, 5.30 P.M.	Aug. 25, 11.30 A.M.
”	”	”	”	”	”	P 6	”	Aug. 25, 12 noon
F 2	”	”	Not	..	0	P 7	”	”
”	”	”	”	..	0	P 8	”	Aug. 25, 12.30 P.M.
F 1	”	”	Exposed	Aug. 20, 11.30 A.M. —5 P.M.	5½	P 9	Aug. 21, 11.30 A.M.	Aug. 24, 12 noon
”	”	”	”	”	”	P 10	”	Aug. 27, 11 A.M.
F 2	”	”	Not	..	0	P 11	”	Aug. 26, 8 A.M.
”	”	”	”	..	0	P 12	”	Aug. 25, 1 P.M.
F 1	”	”	Exposed	Aug. 20, 11.30 A.M.—5 P.M.; Aug. 21, 11.15 A.M.—4 P.M.	10¼	P 13	Aug. 21, 5.15 P.M.	Aug. 27, 11.30 A.M.
”	”	”	”	”	”	P 14	”	Aug. 27, 12 noon
F 2	”	”	Not	..	0	P 15	Aug. 21, 5.30 P.M.	Aug. 26, 7 A.M.
”	”	”	”	..	0	P 16	”	Aug. 28, 11 AM.

Spores in Distilled Water.

Hours of incubation.	Temperature of incubation.	Number of colonies on plate.	Number of bacteria per c.c.	Remarks.	Weather.
94	18—20°	5000	1,950,000	1 c.c. of water from the flask diluted to 10 c.c. with sterile distilled water, and the plate made from a drop of the mixture.	
71	„	6000	2,340,000	„	
71½	„	6000	2,160,000	„	
„	„	5700	2,052,000	„	
114	„	5000	1,700,000	„	Cloud all the time, and a good deal of rain.
114½	„	1000	340,000	Badly levelled plate; anthrax colonies all up at one side.	„
„	„	1700	595,000		
115	,	3700	1,295,000		
72½	„	3350	1,149,000	Two liquefying, twenty-five *large* anthrax, and possibly very many smaller, very numerous small colonies round the edge. Diluted	Flask had been placed on ice overnight, 6 P.M. to 11 A.M.
143½	„	1900	646,000	Diluted. Nine moulds and four other foreign forms.	„
116½	„	140	42,000	Diluted.	„
97½	„	4000	1,200,000	Diluted	Numerous intruders.
138¼	„	353	122,500	Diluted. One mould, forty-two (?) anthrax	Clear sunshine, with clouds occasionally. About three hours sun.
138¾	„	145	50,750	Diluted.	
109½	„	6000	Total 1,920,000	Diluted.	
161½	„	2	640	Diluted	This plate is so abnormal that there was obviously some blunder.

Table O—

Flask.	Contents.	When filled.	Exposed or not.	Time of exposure.	Number of hours exposed.	Number of plate.	When made.	When examined.
F 1	Anthrax spores in distilled water ½ in. deep.	Aug. 20, 11 A.M.	Exposed	Aug. 20, 11.30 A.M.—5 P.M.; Aug. 21, 11.15 AM.—4 P.M.	$10\frac{1}{4}$	P 21	Aug. 22, 2.30 P.M.	Aug. 29, 12 noon
F 2	”	”	Not	..	0	P 22	Aug. 22, 3 P.M.	Aug. 27, 12.30 P.M.
F 2	”	”	”	..	0	P 36	”	”
F 1	”	”	Exposed	Aug. 20, 11.30 A.M.—5 P.M.; Aug. 21, 11.15 A.M.—4 P.M.; Aug. 23, 9 A.M.—4.30 P.M.	$17\frac{3}{4}$	P 35	Aug. 23, 6.30 P.M.	Aug. 29, 12 noon

energetic than has been commonly supposed, and the results also suggest that some spores die off very quickly, even in the dark, when put into sterile water—a fact long known.

On August 18, we started a similar experiment to the last, using Thames water, freshly collected, instead of sterilised distilled water; but this had to be abandoned owing to the difficulties with the rapidly-developing liquefying forms at the temperatures necessary for growing the anthrax. There was nothing in the results to contradict previous experience, but the details are of little value.

§ XX.

On October 6 I exposed a tube of broth, infected with a loopful of colonies β—from gelatine stab-culture—from 9 A.M. to 4 P.M. (seven hours), over a mirror, to the sun; the sky was clear, and sun bright till about 1 P.M., and then duller. An exactly similar tube was wrapped in foil and black paper, and placed by the side of the above.

At 4 P.M. a plate was made from each tube, and incubated at 15° C.; a stab-culture from each was also made and kept at 15° C., and the original tubes were placed at 20—22° C.

Taking the plates first. In forty hours the plate from unexposed tube showed numerous colonies, from 0·5 mm. to 1 mm. diameter,

continued.

Hours of incubation.	Temperature of incubation.	Number of colonies on plate.	Number of bacteria per c.c.	Remarks.	Weather.
165½	18—20°	0 Anthrax	0 Anthrax	Diluted. Six small strangers	Flask on ice, Aug. 21, 9 P.M., to Aug. 22, 12 noon.
117½	,,	990	297,000	Diluted	,,
,,	,,	2	720	,,	,,
137½	,,	20	6,800	,,	One hour sun 3—4 P.M. Flask on ice 3 P.M., Aug. 22, to 9 A.M., Aug. 23.

and quite visible to the unaided eye, the larger ones opening out and swarming vigorously as liquefaction began. A greenish shimmer showed where the colonies were very dense. In sixty-four hours the colonies were running together, and liquefying rapidly, and in eighty-six hours the whole gelatine was liquid and watery.

The plate from the insolated tube, treated in exactly the same way, showed no trace of colonies in forty hours, and only one or two minute colonies, invisible to the unaided eye, could be detected under the microscope in sixty-four hours. Even after seven days the morula-like, dense, granular colonies only showed traces of opening out and the first beginnings of liquefaction.

Of the stab-cultures, that from the covered tube showed the beginnings of growth in forty hours, and had formed a thistle-head funnel of liquefaction in sixty-four hours, which rapidly extended in eighty-eight hours.

That from the insolated tube, on the contrary, showed no trace of growth in forty hours, or even in sixty-four hours to eighty-eight hours.

Of course it may be objected that the difference here was entirely due to the stab-infection having carried in so few living germs in the latter case; but to this it must be replied, that the plates show

Table P.

Flask.	Contents.	When filled.	Exposed or not.	Time of exposure.	Hours exposed.	Number of plate.	When made.	When examined.	Hours of incubation.	Temperature of incubation.	Number of colonies.	Number of bacteria per c.c.	Remarks.
Ax	Anthrax spores in sterile distilled water	Aug. 16, 10 A.M.	Not	..	0	× A I	Aug. 16, 10 A.M.	Aug. 18, 11.20 A.M.	$25\frac{1}{2}$	22·25	23,000	897,000	Liquefied.
„	„	„	Exposed with mirror	Aug. 16, 10 A.M.—4 P.M.	6	× A II	Aug. 16, 4.30 P.M.	Aug. 19, 4 P.M.	$72\frac{1}{2}$	..	117	4,563	
„	„	„	„	„	6	× A II′	„	„	„	..	40	1,360	
Bx	Anthrax spores in sterile distilled water	Aug. 16, 10 A.M.	Not	..	0	× B I	Aug. 16, 10 A.M.	Aug. 18, 11 A.M.	25	..	5,000	170,000	Liquefied and certainly underestimated.
„	„	„	„	..	0	× B II	Aug. 16, 4.30 P.M.	Aug. 18, 5 P.M.	$24\frac{1}{2}$	..	5,900	200,600	
„	„	„	„	Aug. 16, 10 A.M. to 4 P.M.	0	× B II′	„	Aug. 18, 4 P.M.	$23\frac{1}{2}$	..	6,600	228,400	

clearly that the *individual colonies* are weakened by the light-action, and the results with the original tubes (given below) may also be consulted.

Turning now to the original broth cultures, placed at 20—22° C. In eighteen hours the darkened tube was distinctly turbid, whereas that exposed to the sun was perfectly clear; and the same was the case at the end of forty hours. In sixty-four hours both tubes were turbid, but the exposed one far less so than the other. Later on it was impossible to distinguish between them.

It seems to me impossible to avoid the conclusion that the light-action so weakens the metabolism of the insolated cells that they grow and divide more slowly, and dissolve the gelatine more feebly, and possibly this weakening effect is transmitted to the cells to which they give rise by division. As time passes, however, the cells gradually recover their vigour in the dark, and where plenty of food material is accessible.

That this latter statement is true the following experiment proves:—On October 18 I took the two broth-tubes of Colony β, referred to above, both of which had been in the dark, side by side, since October 6. Broth-tubes were exactly similar at the beginning, as we have seen, but one of them had been exposed, on October 6, for seven hours to the sunlight.

On making stab-cultures from these tubes, no difference could be detected between their behaviour, both began to liquefy the gelatine in forty-eight hours in the typical, thistle-head funnel form.

This seems to show clearly, also, that the broth has not been injured as a medium for culture by its exposure to light.

In order to meet the objection that the above results were due to the using of broth-cultures, I repeated them as follows on October 10:—

Two tubes of sterile water, infected from the turbid broth-culture kept in the dark since October 6, were suspended, as already described—one exposed over a mirror to the bright direct sunlight from 10 A.M. to 2 P.M., the other wrapped up, and placed beside it. At 2 P.M. plates and stab-cultures were made from each tube.

The results were the same as before. The plates from the unexposed tubes showed numerous liquefying colonies in forty-eight hours, and were completely liquefied on the third day; the plates from the insolated tube showed only very few colonies, and these not till the third day, and they were denser, more granular, and without any signs of liquefaction for several days. To take a concrete case: a plate made with 1 drop (1/30 c.c.) from the dark tube showed about 5000 colonies in forty-eight hours, and these were already beginning to run; the whole of the gelatine was liquefied in another twenty-four hours; the corresponding plate made with 1 drop (1/24 c.c.)

from the insolated tube showed three colonies only on the third day, and these were very small, more densely granular, and irregular in outline than the normal colonies, and showed no traces of liquefaction even on the fourth day. On the fifth day the liquefaction was beginning, but even after fourteen days one of the three colonies was only just breaking up.

The stab-cultures gave similar results. Those from the darkened tube showed a distinct thistle-head funnel of liquefaction in three days, whereas the feeblest signs that infection had really occurred were all I could get in the same time from the insolated cultures.

Here, again, however, the two sets of tubes gradually become alike, evidently because the at first enfeebled cells gradually regain their vigour, and once more rapidly peptonise the medium.

The experiments with water were repeated on October 12, all the arrangements being as before.

The exposed tube was out from 9 A.M. to 3 P.M. in brilliant sunshine the whole time practically.

After three hours' exposure, a plate was made. That from the dark tube showed colonies, visible only under the microscope, in nineteen hours, and in two days the whole of the gelatine was liquid like water.

The plate from the lighted tube showed no signs until the third day; on the fourth day six colonies had made their appearance, but these only began to soften the gelatine around some days later, and even on the twelfth day two of the six colonies were still circular depressions, though the other four had liquefied the gelatine some distance round.

After six hours' exposure, further plates were made from the above tubes on October 12.

As before, colonies were visible with the lens on the plate made from the dark tube in sixteen hours, and before the end of the second day the whole of the gelatine was liquefied like water.

On the plate from the light tube five slowly-developing colonies had appeared by the fourth day, one of which showed feeble signs of liquefaction next day. But even after twelve days only three of these colonies were vigorously liquefying the gelatine, the other two being still compact and circular, though one of them lay in a slight depression.

Stab-cultures were also made at the end of the six hours' exposure on October 12, with the results as before. In the case of the culture from the dark tube, the funnel of liquefaction had reached the walls of the tube, and liquefied one-eighth of an inch of gelatine in four days, whereas that from the lighted tube, in the same period and side by side, had not even begun to liquefy the gelatine, though the infection had taken.

I also, at the end of the experiment on October 12, cautiously emptied each of the water tubes, and drained it until only a drop remained, and then filled up with sterile broth. After sixteen hours the dark tube was distinctly turbid, whereas no trace of turbidity appeared in the insolated one till after forty-eight hours. Both were equally turbid on the fourth day.

Of course I recognise that this last result, and that obtained with the stab-cultures, is attributable to the *numbers* of still living germs added to the gelatine and broth respectively, and the experiments only go to prove once more, but in a very decisive manner, what mortality the sun had occasioned in the exposed tube—for we must remember, each tube contained practically the same enormous numbers at the start.

§ XXI.

On October 6 a loopful of a markedly liquefying form, marked in my notes as colony θ, was placed in each of two tubes of broth, and placed in the sun from 9 A.M. to 4 P.M. One tube was exposed over a mirror, the other side by side, but wrapped in foil and black paper. The sun shone brightly from 9 to 1, and then was obscured by clouds.

At 4 P.M. a plate and a stab-culture from each tube were made, and the tubes put at 20° to 22° C. in the dark incubator. The plate and stab-cultures were put at 15° C. in the dark.

Taking the broth tubes first. Both were already turbid in eighteen hours, more densely so after forty hours, so that no difference between them could be detected.

Of the stab-cultures, that from the dark tube had taken in forty hours, while the one from the lighted tube showed no signs.

In sixty-four hours the non-illuminated culture had developed a thistle-head liquefaction funnel, whereas no trace was visible in the other. In eighty-eight hours the liquefaction had proceeded rapidly in the former tube; only one feeble colony was visible in the tube from the insolated broth. In the course of a week or so ɪo further difference could be made out.

With regard to the plates. That from the insolated tube showed no trace until sixty-four hours had passed, when two or three colonies $\frac{1}{5}$ to $\frac{1}{2}$ mm. diameter were seen. In eighty-six hours these were somewhat like young anthrax colonies, each with a slight depression. On the sixth day liquefaction of the gelatine was slowly evincing itself.

The plate from the dark tube showed evident colonies, $\frac{2}{5}$ to $\frac{1}{2}$, and even 1 mm. in diameter, in forty hours; and in sixty-four hours they averaged 2 to 5 mm. in diameter, and were liquefying rapidly. These circular colonies were less rapid than those of colony β at tho

same time, however, and whiter in colour. In eighty-eight hours the whole of the gelatine was completely liquefied.

§ XXII.

On October 30 about 200 c.c. of sterilised Thames water were strongly infected with the bacillus called *B. arborescens* by Frankland, taken from a vigorous culture.

The infected liquid was divided equally into two Erlenmeyer flasks, one of which was at once wrapped in tin-foil and black paper. The other was supported above a plane mirror, and placed so as to obtain the maximum amount of direct sunshine available from 10 A.M. to 5 P.M. that day; from 10 to 1 the sunshine was hot and bright, and a control flask showed that the water rose to nearly 20° C., but the afternoon was dull and cloudy, and the temperature fell to 12° C. The temperature in the covered flask, placed side by side with the exposed one, rose and fell so nearly exactly with that of the latter, that no stress can be laid on the difference.

Before commencing the experiment at 10 A.M., sample plates were made of the infected water; and at 5 P.M. two plates were made from each flask—exposed and unexposed.

The plates from the freshly infected material showed the usual rapidly growing, loose, thread-like colonies in forty-eight hours, and in three days the gelatine was entirely liquefied to a watery fluid.

The plates from the flask, wrapped in foil and paper, and sheltered from the direct rays of the sun, behaved similarly, the colonies being a trifle more compact in shape, but equally rapid in liquefying the gelatine completely.

But the plates from the exposed flask differed from the first onwards from those not insolated. Thus no colonies were visible in forty-eight hours, a period during which the plates from the unexposed flasks exhibited numerous typical colonies; the colonies appeared here twenty-four hours later, clearly showing the effects of inhibition due to the light.

Secondly, when the colonies did make their appearance they were more compact, and instead of shooting out in all directions and covering the plate with a meshwork of fine branches, rapidly liquefying the gelatine, as in the case of the unexposed specimens, the mode of growth was so affected that on the fourth day they had developed into beautifully circular yellowish colonies, zoned, and radially striated, and only just softening the gelatine. It was not, indeed, till the sixth day that liquefaction set in generally.

I explain the differences as follows. The light retards the growth of the living bacilli, owing to some action on their protoplasm which induces interference with the metabolic processes on which growth

depends; this causes the cell-chains to be so modified in length, direction, and rapidity of development, that the colony formed from the insolated germ is weaker and more condensed, or compact, than normally. Thus result the differences in the naked eye characters of the colonies, which may go so far that the total aspect on the gelatine plate is altered.

Some clue to the action may perhaps be got eventually by following up the fact that one consequence of the light action is to weaken the enzyme action of the bacterium—for the enfeebled liquefying power is an expression of enfeebled enzyme power—either by so altering the protoplasmic machinery that less enzyme is secreted, or by so acting on the enzyme that its power of converting the medium is altered. The lessened enzyme power of course implies less power to obtain its food from the medium, and so the progeny developed from the germ started with are also feebler than the normal one.

In the cases quoted, however, the colony gradually becomes more normal as regards its enzyme power, especially in the dark, because the successively developed new cells become stronger and stronger as they are fed by the nutrient gelatine, and at last the differences are equalised.

The only source of error in the above conclusion that I could think of, was the possibility that the rapid running of the colonies into thin filaments in the first case is facilitated by the quicker liquefaction of the gelatine, and that this liquefaction is, in turn, more rapid, because there are so many more colonies per drop in the unexposed flasks, because the majority of the bacilli in the exposed flasks are killed.

I accordingly made plates in which I diluted the samples from the unexposed flasks five, ten, and even twenty times as much as the samples from the exposed flasks, and so brought the numbers of colonies on each plate approximately equal. Of course it may be replied here that the differences of dilution may bring about differences in development; but experience shows that increased dilution tends to *inhibition* of colonies, and so I think the fact that I still get the differences in the colonies already described, strengthens rather than weakens the evidence that the alteration in the character of the colonies from exposed flasks is really due to the action of the light.

On November 7, which turned out a beautifully fine day, with clear blue sky and bright sun, tubes of sterile distilled water were infected with cultures of a yellow bacillus marked *, a large white one marked ⟐, and a violet bacillus, common in the Thames. In each case, two similar tubes were prepared, exactly alike, one of which was exposed over a mirror, from 10.30 A.M. to 3.30 P.M.; while the other was wrapped in foil and black paper, and placed by the side of the exposed one. The temperature recorded in control tubes was 10—12° C.

At 4 P.M., after five hours' exposure, plates were made, and incubated at 15° C.

Taking the violet bacillus first. Nothing appeared on either of the two plates made from the exposed tubes, although they were kept till November 24, *i.e.*, seventeen days. On the plate made from the dark tube, two white colonies were visible in forty-eight hours, and on the 13th—*i.e.*, after six days—three colonies were seen. These remained white until December 1, when one of them began to show the violet line. On December 6 this was more pronounced.

The experiment, therefore, showed negative results only, and I regard it as probable that the mere immersion in water injures the bacillus. On the other hand, it is possible I did not use sufficient material in making the plates.

Thus the plates of exposed tube = 1/28th c.c. of 7/28 dilution, and that of dark one = 1/27th c.c. of 1/27 dilution, thus giving only 2187 per c.c. even in the unexposed tube, making it probable that the last suggestion is the right one.

Now, as regards the yellow bacillus, Colony *. Of the two plates made from the insolated tube, one was contaminated, and yielded no results, except that plenty of colonies appeared; the other failed utterly. The plate from the dark tube showed innumerable typical colonies, and was completely liquefied on the fourth day.

The experiments consequently must be regarded as negative.

The plates of Colony ⟐ behaved as follows. That from the dark tube gave a typical series of colonies—about 500 = $500 \times 28 \times 28$ = 392,000 per c.c., softening the gelatine in two days.

That from the lighted tube gave far fewer—about $100 \times 25 \times 5$ = 12,500 per c.c.—colonies, and these smaller, showing evident retardation; otherwise no results.

On November 12 the above experiment was repeated with Colony *, Colony ⫽, and the rosy-red bacillus δ, exactly as before. The day was cold and bright, though some haze appeared after 1 P.M. Exposure 10.30 to 3.30 as before, and thermometers = 10—12° C.

The plates of Colony * behaved as follows. That from the dark tube was liquefied by numerous colonies, which appeared on the second day. On the fourth day all the gelatine was liquefied.

The plates (two) made from the insolated tube only showed slight retardation, and liquefied also on the fourth day, though more slowly.

No difference between illuminated and dark tubes could be made out in the case of Colony ⫽, except slight retardation on the plates from the former.

The results with Colony δ were still more indecisive, and I could not draw any conclusions as to distinct light action.

It appears probable that considerable differences will be found between the various forms in this respect.

§ XXIII.

In the following tables I give the determinations of the numbers of all bacteria found in three series of analyses of samples of Thames water in August, October, and December, 1893. As will be seen, they confirm the results of other observers, in so far that they show that the number per c.c. is considerably greater in winter than in summer.

There is nothing special to note as regards the methods employed, which were those ordinarily in use, beyond the fact that we counted *every* colony on each plate, instead of estimating the averages from a few squares.

The greatest care was taken to have the gelatine made up exactly alike in every case; further information as to details is supplied by the tables.

Table Q.—Number of Bacteria in 1 c.c. Thames Water in August, 1893.

Date.	Plate.	Age of water.	Temperature of incubation.	Time incubated.	Dilute or not.	No. of drops.	Colonies on plate.	Calculated No. of bacteria per 1 c.c.	Averages.
		° C.	° C.	days.		c.c.			
12.8.93	A 1	1 h. at 15—18	12—15	2	not	$\frac{1}{39}$	40	1560	
”	B 1	”	”	”	”	$\frac{1}{34}$	50	1700	1446
”	C 1	”	”	”	”	$\frac{1}{36}$	30	1080	
”	A 1	”	”	3	”	$\frac{1}{39}$	95	3705	2680
”	C 1	”	”	”	”	$\frac{1}{36}$	46	1656	
14.8.93	A 1′	”	15—18	2	”	$\frac{1}{39}$	45	1755	
”	A 1‴	”	”	”	”	$\frac{1}{39}$	36	1404	
”	B 1′	”	”	”	”	$\frac{1}{34}$	20	680	1434
”	B 1‴	”	”	”	”	$\frac{1}{34}$	64	2176	
”	C 1″	”	”	”	”	$\frac{1}{34}$	34	1156	
22.8.93	P. 17	”	16—18	2	”	$\frac{1}{32}$	25	800	
”	P. 18	”	”	”	”	$\frac{1}{32}$	44	1408	1104
”	P. 19	”	”	”	”	$\frac{1}{34}$	22	748	
”	P. 20	”	”	”	”	$\frac{1}{34}$	43	1462	
”	P. 17	”	”	3	”	$\frac{1}{32}$	26	832	
”	P. 18	”	”	”	”	$\frac{1}{32}$	60	1920	1189
”	P. 19	”	”	”	”	$\frac{1}{34}$	24	816	
24.8.93	P. 37	”	16—18	4	$\frac{1}{10}$	$\frac{1}{33}$	6	1980	
”	P. 38	”	”	”	”	”	5	1650	1980
”	P. 39	”	”	”	”	”	4	1320	
”	P. 40	”	”	”	”	”	9	2970	
25.8.93	P. 45	”	”	3	not	$\frac{1}{34}$	49	1666	
”	P. 46	”	”	”	”	”	38	1292	1530
”	P. 47	”	”	”	”	”	41	1394	
”	P. 48	”	”	”	”	”	52	1768	

25.8.93	P. 45	,,	,,	5	,,	$\frac{1}{34}$	100	3400	3356
,,	P. 46	,,	,,	,,	,,	,,	64	2716	
,,	P. 47	,,	,,	,,	,,	,,	79	2686	
,,	P. 48	,,	,,	,,	,,	,,	136	4624	
28.8.93	P. 55	,,	,,	3	$\frac{1}{10}$	$\frac{1}{33}$	5	1650	1555
,,	P. 56	,,	,,	,,	,,	,,	4	1320	
,,	P. 57	,,	,,	,,	,,	,,	4	1320	
,,	P. 58	,,	,,	,,	,,	,,	6	1980	
,,	P. 59	,,	,,	,,	,,	,,	6	1980	
,,	P. 61	,,	,,	,,	,,	,,	4	1320	
,,	P. 62	,,	,,	,,	,,	,,	4	1320	
28.8.93	P. 56	,,	,,	4	$\frac{1}{10}$	$\frac{1}{33}$	11	3630	3300
,,	P. 57	,,	,,	,,	,,	,,	7	2310	
,,	P. 58	,,	,,	,,	,,	,,	10	3300	
,,	P. 59	,,	,,	,,	,,	,,	12	3960	
28.8.93	P. 56	,,	,,	5	$\frac{1}{10}$	$\frac{1}{33}$	14	4620	3795
,,	P. 57	,,	,,	,,	,,	,,	7	2310	
,,	P. 58	,,	,,	,,	,,	,,	13	4290	
,,	P. 59	,,	,,	,,	,,	,,	12	3960	
29.8.93	P. 64	,,	,,	2	not	$\frac{1}{34}$	6	204	136
,,	P. 67	,,	,,	,,	,,	,,	2	68	
30.8.93	78	1 h. at 16—18	16—18	3	$\frac{1}{10}$	$\frac{1}{30}$	5	1500	1350
,,	79	,,	,,	,,	,,	,,	1	300	
,,	80	,,	,,	,,	,,	,,	3	900	
,,	81	,,	,,	,,	,,	,,	4	1200	
,,	82	,,	,,	,,	,,	,,	7	2100	
,,	83	,,	,,	,,	,,	,,	3	900	
,,	84	,,	,,	,,	,,	,,	10	3000	
,,	85	,,	,,	,,	,,	,,	3	900	
30.8.93	79	1 h. at 16—18	16—18	4	$\frac{1}{10}$	$\frac{1}{30}$	1	300	1713
,,	80	,,	,,	,,	,,	,,	3	900	
,,	81	,,	,,	,,	,,	,,	9	2700	
,,	82	,,	,,	,,	,,	,,	7	2100	
,,	83	,,	,,	,,	,,	,,	5	1500	
,,	84	,,	,,	,,	,,	,,	10	3000	
,,	85	,,	,,	,,	,,	,,	5	1500	

Table Q—*continued.*

Date.	Plate.	Age of water.	Temperature of incubation.	Time incubated.	Dilute or not.	No. of drops.	Colonies on plate.	Calculated No. of bacteria per 1 c.c.	Averages.
		° C.	° C.	days.		c.c.			
30.8.93	78	1 h. at 16—18	16—18	6	$\frac{1}{10}$	$\frac{1}{30}$	6	1800	3037
"	79	"	"	"	"	"	2	600	
"	80	"	"	"	"	"	4	1200	
"	81	"	"	"	"	"	15	4500	
"	82	"	"	"	"	"	14	4200	
"	83	"	"	"	"	"	10	3000	
"	84	"	"	"	"	"	21	6300	
"	85	"	"	"	"	"	9	2700	

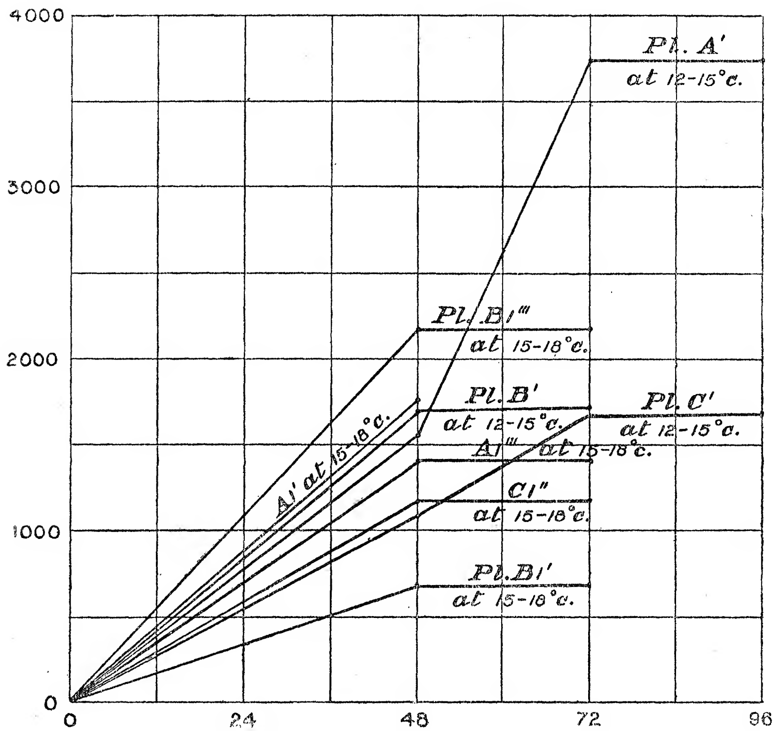


DIAGRAM I.—Curves of Table Q, showing total bacteria at 12—15° C. and 15—18° C. in August. Abscissæ = hours of incubation of plates; ordinates = numbers of colonies per 1 c.c.

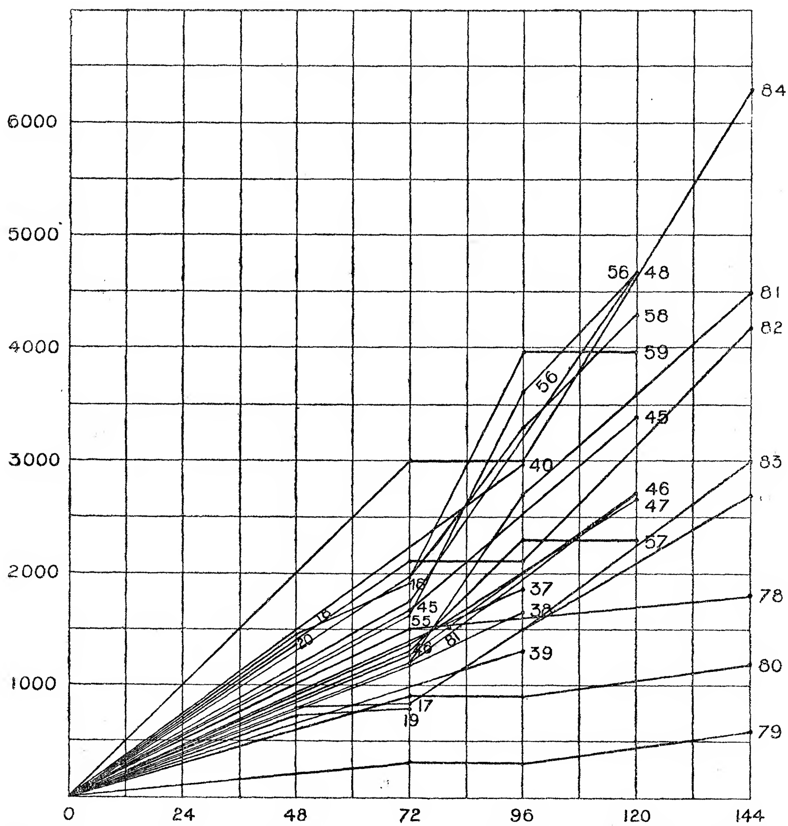


DIAGRAM II.—Curves of Table Q, showing total bacteria at 16—18° C. in August. Abscissæ = hours of incubation of plates; ordinates = numbers of colonies of bacteria per 1 c.c.

Quantitative Analysis of Thames Water in August, 1893 (*Table Q*).

If we analyse these August numbers, we get as the average of 14 two-days' plates, 1157; of 24 three-days' plates, we have 1530; of 15 four-days' plates, we have 2208; of 8 five-days' plates, we get 3575; and of 8 six-days' plates, we have 3037, as the approximate averages. Of course these numbers can only be regarded as approximations, but I think they are of use as indicating what might be looked for if the necessary large series of observations could be made over several years; and they certainly serve to put us on our guard against placing too much confidence in the gelatine method unless a great number of observations are made, extending over a long period. Of course the great difficulty to be contended against is that of keeping the plates long enough; if only *one* badly liquefying form is present, it ruins the plates before the slowly developing ones germinate out.

Quantitative Analysis of Thames Water in October, 1893 (*Table R*).

The following table gives the details of the analysis for October, and again I have recorded all the points. The chief feature of interest is the much longer time during which the plates could be cultivated, in spite of the prevalence of liquefying forms, where care was taken to dilute sufficiently.

Table R.—Number of Bacteria in 1 c.c. Thames Water in October, 1893.

Date.	Plate.	Age of water.	Temperature of incubation.	Time incubated.	Diluted or not.	No. of drops.	Colonies on plate.	Calculated No. of bacteria per 1 c.c.	Averages.	Total average.
		° C.	° C.	hours.		c.c.				
21 10.93	E	½ hour at 15	16—17	24	$\frac{1}{5}$	$\frac{1}{27}$	1	135		
”	F	”	”	”	”	”	1	135	169	
”	G	”	”	”	”	”	2	270		
”	H	”	”	”	”	”	1	135		317
”	L	”	”	”	$\frac{1}{3}$	$\frac{1}{27}$	3	243		
”	M	”	”	”	”	”	10	810	466	
”	N	”	”	”	”	”	3	243		
”	P	”	”	”	”	$\frac{2}{27}$	14	567		
”	E	”	”	48	$\frac{1}{5}$	$\frac{1}{27}$	11	1,485		
”	F	”	”	”	”	”	9	1,115	1,494	
”	G	”	”	”	”	”	14	1,890		
”	H	”	”	”	”	”	11	1,485		1,876
”	L	”	”	”	$\frac{1}{3}$	$\frac{1}{27}$	18	1,458		
”	M	”	”	”	”	”	32	2,592	2,258	
”	N	”	”	”	”	”	26	2,106		
”	P	”	”	”	”	$\frac{2}{27}$	71	2,875		
”	E	”	”	72	$\frac{1}{5}$	$\frac{1}{27}$	17	2,295		
”	F	”	”	”	”	”	23	3,405	2,639	
”	G	”	”	”	”	”	21	2,830		
”	H	”	”	”	”	”	15	2,025		3,309
”	L	”	”	”	$\frac{1}{3}$	$\frac{1}{27}$	43	3,483		
”	M	”	”	”	”	”	49	3,969	3,979	
”	N	”	”	”	”	”	51	4,131		
”	P	”	”	”	”	$\frac{2}{27}$	107	4,333		
”	E	”	”	96	$\frac{1}{5}$	$\frac{1}{27}$	27	3,645		
”	F	”	”	”	”	”	30	4,050	4,151	
”	G	”	”	”	”	”	40	5,400		
”	H	”	”	”	”	”	26	3,510		4,920
”	L	”	”	”	$\frac{1}{3}$	$\frac{1}{27}$	64	5,184		
”	M	”	”	”	”	”	75	6,075	5,690	
”	N	”	”	”	”	”	65	5,265		
”	P	”	”	”	”	$\frac{2}{27}$	154	6,237		

,,	E	,,	,,	120	$\frac{1}{5}$	$\frac{1}{27}$	34	4,590	5,265	6,692
,,	F	,,	,,	,,	,,	,,	44	5,940		
,,	G	,,	,,	,,	,,	,,	49	6,615		
,,	H	,,	,,	,,	,,	,,	29	3,915		
,,	L	,,	,,	,,	$\frac{1}{3}$	$\frac{1}{27}$	105	8,505	8,120	
,,	M	,,	,,	,,	,,	,,	104	8,424		
,,	N	,,	,,	,,	,,	,,	86	6,966		
,,	P	,,	,,	,,	,,	$\frac{2}{27}$	212	8,586		
,,	E	,,	,,	144	$\frac{1}{5}$	$\frac{1}{27}$	34	4,590	5,265	6,905
,,	F	,,	,,	,,	,,	,,	44	5,940		
,,	G	,,	,,	,,	,,	,,	49	6,615		
,,	H	,,	,,	,,	,,	,,	29	3,915		
,,	L	,,	,,	,,	$\frac{1}{3}$	$\frac{1}{27}$	108	8,748	8,545	
,,	M	,,	,,	,,	,,	,,	104	8,424		
,,	N	,,	,,	,,	,,	,,	101	8,181		
,,	P	,,	,,	,,	,,	$\frac{2}{27}$	218	8,829		
,,	E	,,	,,	168	$\frac{1}{5}$	$\frac{1}{27}$	35	4,725	5,670	7,209
,,	F	,,	,,	,,	,,	,,	48	6,480		
,,	G	,,	,,	,,	,,	,,	56	7,560		
,,	H	,,	,,	,,	,,	,,	29	3,915		
,,	L	,,	,,	,,	$\frac{1}{3}$	$\frac{1}{27}$	108	8,748	8,748	
,,	M	,,	,,	,,	,,	,,	111	8,991		
,,	N	,,	,,	,,	,,	,,	105	8,505		
,,	A	,,	20—22	24	$\frac{1}{5}$	$\frac{1}{27}$	3	405	742	1,120
,,	B	,,	,,	,,	,,	,,	5	675		
,,	C	,,	,,	,,	,,	,,	4	540		
,,	D	,,	,,	,,	,,	,,	10	1,350		
,,	I	,,	,,	,,	$\frac{1}{3}$	$\frac{1}{27}$	20	1,620	1,498	
,,	J	,,	,,	,,	,,	,,	15	1,215		
,,	K	,,	,,	,,	,,	,,	18	1,458		
,,	O	,,	,,	,,	,,	$\frac{2}{27}$	42	1,701		
,,	A	,,	,,	48	$\frac{1}{5}$	$\frac{1}{27}$	12	1,620	2,464	2,884
,,	B	,,	,,	,,	,,	,,	13	1,755		
,,	C	,,	,,	,,	,,	,,	25	3,375		
,,	D	,,	,,	,,	,,	,,	23	3,108		
,,	I	,,	,,	,,	$\frac{1}{3}$	,,	40	3,240	3,304	
,,	J	,,	,,	,,	,,	,,	41	3,321		
,,	K	,,	,,	,,	,,	,,	52	3,212		
,,	O	,,	,,	,,	,,	$\frac{2}{27}$	85	3,442		

Table R—*continued.*

Date.	Plate.	Age of water.	Temperature of incubation.	Time incubated.	Diluted or not.	No. of drops.	Colonies on plate.	Calculated No. of bacteria per 1 c.c.	Averages.	Total average.
		° C.	° C.	hours.		c.c.				
20.10.93	A	½ hour at 15	20—22	72	$\frac{1}{5}$	$\frac{1}{27}$	15	2,025	3,571	4,696
”	B	”	”	”	”	”	20	2,700		
”	C	”	”	”	”	”	41	5,508		
”	D	”	”	”	”	”	30	4,050		
”	I	”	”	”	” $\frac{1}{3}$	” $\frac{1}{27}$	80	6,480	5,822	
”	K	”	”	”	”	”	76	6,156		
”	J	”	”	”	”	”	74	5,994		
”	O	”	”	”	”	” $\frac{2}{27}$	116	4,657		
”	A	”	”	” 96	” $\frac{1}{5}$	$\frac{1}{27}$	29	3,915	5,737	7,546
”	B	”	”	”	”	”	52	7,020		
”	C	”	”	”	”	”	50	6,750		
”	D	”	”	”	”	”	39	5,265		
”	I	”	”	”	” $\frac{1}{3}$	” $\frac{1}{27}$	128	10,368	9,355	
”	K	”	”	”	”	”	103	8,343		
”	A	”	”	” 120	” $\frac{1}{5}$	” $\frac{1}{27}$	34	4,590	6,885	9,963
”	B	”	”	”	”	”	60	8,100		
”	C	”	”	”	”	”	55	7,425		
”	D	”	”	”	”	”	55	7,425		
”	I	”	”	”	” $\frac{1}{3}$	” $\frac{1}{27}$	157	12,717	13,041	
”	K	”	”	”	”	”	165	13,365		
”	A	”	”	” 144	” $\frac{1}{5}$	” $\frac{1}{27}$	45	6,075	7,830	12,015
”	B	”	”	”	”	”	71	9,585		
”	I	”	”	”	” $\frac{1}{3}$	” $\frac{1}{27}$	190	15,390	16,200	
”	K	”	”	”	”	”	210	17,010		
”	A	”	”	” 168	” $\frac{1}{5}$	” $\frac{1}{27}$	56	7,560	9,450	13,027
”	B	”	”	”	”	”	84	11,340		
”	I	”	”	”	” $\frac{1}{3}$	” $\frac{1}{27}$	200	16,200	16,605	
”	K	”	”	”	”	”	210	17,010		

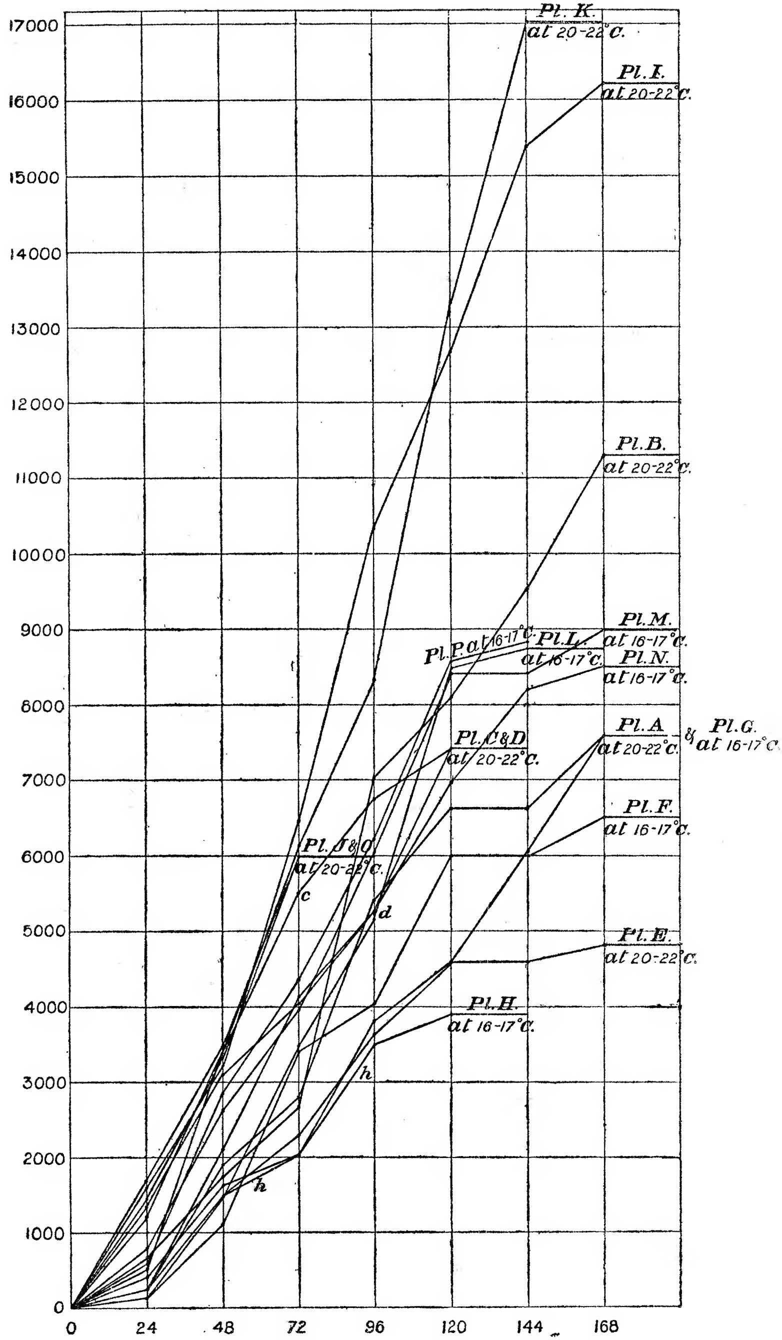


Diagram III.—Curves of Table R, showing total bacteria at 16—17° and 20—22° C. in October. Abscissæ = hours of incubation; ordinates = numbers of colonies per 1 c.c.

Quantitative Analysis of Thames Water in December.

On December 27, 1893, a cloudy and misty cold morning following on a series of bright sunny cold days, a sample of Thames water was taken at 11 A.M., and immediately conveyed to the laboratory, and plates made as follows. The water on collection was at nearly 8° C.

Twenty-two plates in all were made, of which twelve were incubated at 8—12° C., the temperature of the laboratory, and ten at 18—20° C. in the incubator. There were a good many changes of temperature from day to day in the case of the former, though but slight and slow alterations in the latter case.

The following tables give the results of the plate cultures, every colony visible with the hand lens being counted each day as before; and this time the low temperature cultures were continued for 240 hours (ten days), in order to see how far the numbers would eventually approximate to those of the higher temperature plates.

An innovation was made in the case of the plates marked R_1R_2, S_1S_2, T_1T_2. Here I used 1 drop of the Thames water between each pair of plates. The drop was allowed to fall as usual into the tube of gelatine, then a second tube of sterile gelatine emptied into the infected tube; then the contents were distributed rapidly into two plates, the countings of which must be taken together as is done in the table. It is noteworthy how much higher the final numbers are in these plates as compared with others, no doubt chiefly owing to the more complete separation of the bacteria from which the colonies arise, and to their having more space to develope in.

Table S.—Number of Bacteria in 1 c.c. Thames Water in December, 1893.

Date.	Plate.	Age of water.	Temperature of incubation.	Time incubated.	Diluted or not.	Number of drops.	Colonies on plate.	Calculated number of bacteria per 1 c.c.	Averages.	Total average.	
				hours.		c.c.					
Dec. 28, 1893	*a*	½ hour at 8 to 10°	8—12° C.	24	not	$\frac{1}{29}$	1	29	29	7	6
,,	*b*	,,	,,	,,	,,	,,	0	0			
,,	*e*	,,	,,	,,	$\frac{1}{3}$	$\frac{1}{27}$	0	0	0		
,,	*f*	,,	,,	,,	,,	$\frac{1}{27}$	0	0			
,,	*i*	,,	,,	,,	$\frac{1}{5}$	$\frac{1}{98}$	0	0	0		
,,	*j*	,,	,,	,,	,,	,,	0	0			
,,	*m*	,,	,,	,,	,,	$\frac{2}{28}$	0	0	0		
,,	*n*	,,	,,	,,	,,	,,	0	0			
,,	R_1	,,	,,	,,	not	$\frac{1}{27}$	0	0	0	0	
,,	R_2	,,	,,	,,	,,						
,,	S_1	,,	,,	,,	,,	$\frac{1}{27}$	0	0			
,,	S_2	,,	,,	,,	,,						
,,	*a*	,,	,,	48	,,	$\frac{1}{29}$	20	580	609	600	520
,,	*b*	,,	,,	,,	,,	,,	22	638			
,,	*e*	,,	,,	,,	$\frac{1}{3}$	$\frac{1}{27}$	6	486	576		
,,	*f*	,,	,,	,,	,,	$\frac{1}{27}$	8	648			
,,	*i*	,,	,,	,,	$\frac{1}{5}$	$\frac{1}{28}$	7	980	910		
,,	*j*	,,	,,	,,	,,	,,	6	840			
,,	*m*	,,	,,	,,	,,	$\frac{2}{28}$	3	210	315		
,,	*n*	,,	,,	,,	,,	,,	6	420			
,,	R_1	,,	,,	,,	not	$\frac{1}{27}$	24	648	440	440	
,,	R_2	,,	,,	,,	,,						
,,	S_1	,,	,,	,,	,,	$\frac{1}{27}$	27	432			
,,	S_2	,,	,,	,,	,,						
,,	*a*	,,	,,	72	,,	$\frac{1}{29}$	49	1,421	1,464	1,464	1,464
,,	*b*	,,	,,	,,	,,	,,	52	1,508			

Table S—*continued.*

Date.	Plate.	Age of water.	Temperature of incubation.	Time incubated.	Diluted or not.	Number of drops.	Colonies on plate.	Calculated number of bacteria per 1 c.c.	Averages.	Total average.	
				hours.		c.c.					
Dec. 28, 1893	*e*	½ hour at 8–10° C.	8—12° C.	”	$\frac{1}{3}$	$\frac{1}{27}$	15	1,214	1,093	1,466	1,610
”	*f*	”	”	”	”	$\frac{1}{27}$	12	972			
”	*i*	”	”	”	$\frac{1}{5}$	$\frac{1}{28}$	12	1,680	1,750		
”	*j*	”	”	”	”	”	13	1,820			
”	*m*	”	”	”	”	$\frac{2}{28}$	21	1,470	1,155		
”	*n*	”	”	”	”	”	12	840			
”	R_1	”	”	”	not	$\frac{1}{27}$	70	1,890	1,755	1,755	
”	R_2	”	”	”	”						
”	S_1	”	”	”	”	$\frac{1}{27}$	60	1,620			
”	S_2	”	”	”	”						
”	*a*	”	”	96	”	$\frac{1}{20}$	80	2,320	2,436	2,441	3,346
”	*b*	”	”	”	”	”	88	2,552			
”	*e*	”	”	”	$\frac{1}{3}$	$\frac{1}{27}$	31	2,510	2,470		
”	*f*	”	”	”	”	$\frac{1}{27}$	30	2,430			
”	*i*	”	”	”	$\frac{1}{5}$	$\frac{1}{28}$	16	2,240	2,800		
”	*j*	”	”	”	”	”	24	3,360			
”	*m*	”	”	”	”	$\frac{2}{28}$	31	2,170	2,060		
”	*n*	”	”	”	”	”	28	1,960			
”	R_1	”	”	”	not	$\frac{1}{27}$	173	4,671	4,252	4,252	
”	R_2	”	”	”	”						
”	S_1	”	”	”	”	$\frac{1}{27}$	142	3,834			
”	S_3	”	”	”	”						

”	*a*	”	”	120	”	$\frac{1}{29}$	105	3,045	3,915	3,926	5,358
”	*b*	”	”	”	”	”	165	4,785			
”	*e*	”	”	”	$\frac{1}{3}$	$\frac{1}{27}$	51	4,132	3,645		
”	*f*	”	”	”	”	$\frac{1}{27}$	39	3,159			
”	*i*	”	”	”	$\frac{1}{5}$	$\frac{1}{28}$	29	4,060	4,200		
”	*j*	”	”	”	”	”	31	4,340			
”	*m*	”	”	”	”	$\frac{2}{28}$	56	4,040	3,945		
”	*n*	”	”	”	”	”	55	3,850			
”	R_1	”	”	”	not	$\frac{1}{27}$	277	7,479	6,790	6,790	
”	R_2	”	”	”	”						
”	S_1	”	”	”	”	”	226	6,102			
”	S_2	”	”	”	”						
”	*a*	”	”	144	”	$\frac{1}{29}$	182	4,278	5,329		8,856
”	*b*	”	”	”	”	”	220	6,380			
”	*e*	”	”	”	$\frac{1}{3}$	$\frac{1}{27}$	70	5,670	5,548	6,481	
”	*f*	”	”	”	”	$\frac{1}{27}$	67	5,427			
”	*i*	”	”	”	$\frac{1}{5}$	$\frac{1}{28}$	60	8,400	7,490		
”	*j*	”	”	”	”	”	47	6,580			
”	*m*	”	”	”	$\frac{1}{5}$	$\frac{2}{28}$	93	6,510	6,405		
”	*n*	”	”	”	”	”	90	6,300			
”	R_1	”	”	”	not	$\frac{1}{27}$	403	10,881	11,232	11,232	
”	R_2	”	”	”	”						
”	S_1	”	”	”	”	$\frac{1}{27}$	429	11,583			
”	S_2	”	”	”	”						
”	*a*	”	”	16	”	$\frac{1}{29}$	293	8,497	10,254	9,978	29,385
”	*b*	”	”	”	”	”	414	12,006			
”	*e*	”	”	”	$\frac{1}{3}$	$\frac{1}{27}$	119	9,638	10,758		
”	*f*	”	”	”	”	$\frac{1}{27}$	148	11,988			
”	*i*	”	”	”	$\frac{1}{5}$	$\frac{1}{28}$	85	11,900	10,010		
”	*j*	”	”	”	”	”	58	8,120			
”	*m*	”	”	”	”	$\frac{2}{28}$	136	9,520	8,890		
”	*n*	”	”	”	”	”	118	8,260			
”	R_1	”	”	”	not	$\frac{1}{27}$	650	17,550	18,859	18,859	
”	R_2	”	”	”	”						
”	S_1	”	”	”	”	$\frac{1}{27}$	747	20,169			
”	S_2	”	”	”	”						

Table S—*continued.*

Date.	Plate.	Age of water.	Temperature of incubation.	Time incubated.	Diluted or not.	Number of drops.	Colonies on plate.	Calculated number of bacteria per 1 c.c.	Averages.	Total average.	
				hours.		c.c.					
Dec. 28, 1893	*a*	½ hour at 8–10° C.	8—12° C.	192	not	$\frac{1}{29}$	312	9,048	10,614	11,017	12,610
"	*b*	"	"	"	"	"	420	12,180			
"	*e*	"	"	"	$\frac{1}{3}$	$\frac{1}{27}$	125	10,125	11,056		
"	*f*	"	"	"	"	"	148	11,988			
"	*i*	"	"	"	$\frac{1}{5}$	$\frac{1}{28}$	102	14,280	11,550		
"	*j*	"	"	"	"	"	63	8,820			
"	*m*	"	"	"	"	$\frac{2}{28}$	136	9,520	10,850		
"	*n*	"	"	"	"	"	174	12,180			
"	R_1	"	"	"	not	$\frac{1}{27}$	650	17,550	18,981	18,981	
"	R_2	"	"	"	"						
"	S_1	"	"	"	"	$\frac{1}{27}$	756	20,412			
"	S_2	"	"	"	"						
"	*a*	"	"	216	"	$\frac{1}{29}$	425	12,325	13,876	14,303	15,954
"	*b*	"	"	"	"	"	532	15,428			
"	*e*	"	"	"	$\frac{1}{3}$	$\frac{1}{27}$	187	15,141	16,561		
"	*f*	"	"	"	"	"	222	17,982			
"	*i*	"	"	"	$\frac{1}{5}$	$\frac{1}{28}$	105	14,700	11,900		
"	*j*	"	"	"	"	"	65	9,100			
"	*m*	"	"	"	"	$\frac{2}{28}$	193	13,510	14,875		
"	*n*	"	"	"	"	"	232	16,240			
"	R_1	"	"	"	not	$\frac{1}{27}$	781	21,087	22,410	22,410	
"	R_2	"	"	"	"						
"	S_1	"	"	"	"	$\frac{1}{27}$	842	22,734			
"	S_2	"	"	"	"						

”	*a*	”	”	240	”	$\frac{1}{29}$	460	13,340	14,471	15,146	16,714
”	*b*	”	”	”	”	”	538	15,602			
”	*e*	”	”	”	$\frac{1}{3}$	$\frac{1}{27}$	195	15,795	16,888		
”	*f*	”	”	”	”	”	222	17,982			
”	*i*	”	”	”	$\frac{1}{5}$	$\frac{1}{28}$	128	17,920	13,860		
”	*j*	”	”	”	”	”	70	9,800			
”	*m*	”	”	”	”	$\frac{2}{28}$	200	14,000	15,365		
”	*n*	”	”	”	”	”	239	16,730			
”	R_1	”	”	”	not	$\frac{1}{27}$	794	21,438	22,986	22,986	
”	R_2	”	”	”	”						
”	S_1	”	”	”	”	$\frac{1}{27}$	905	24,435			
”	S_2	”	”	”	”						
”	*c*	”	18—20° C.	24	not	$\frac{1}{29}$	6	174	188	304	269
”	*d*	”	”	”	”	”	7	203			
”	*g*	”	”	”	$\frac{1}{3}$	$\frac{1}{27}$	5	405	324		
”	*h*	”	”	”	”	”	3	243			
”	*k*	”	”	”	$\frac{1}{5}$	$\frac{1}{28}$	2	280	420		
”	*l*	”	”	”	”	”	4	560			
”	*o*	”	”	”	”	$\frac{2}{28}$	5	350	280		
”	*p*	”	”	”	”	”	3	210			
”	T_1	”	”	”	not	$\frac{1}{27}$	5	135	135		
”	T_2	”	”	”	”						
”	*c*	”	”	48	”	$\frac{1}{29}$	88	2,552	2,697	2,302	2,597
”	*d*	”	”	”	”	”	98	2,843			
”	*g*	”	”	”	$\frac{1}{3}$	$\frac{1}{27}$	26	2,106	2,065		
”	*h*	”	”	”	”	”	25	2,025			
”	*k*	”	”	”	$\frac{1}{5}$	$\frac{1}{28}$	10	1,400	1,540		
”	*l*	”	”	”	”	”	12	1,680			
”	*o*	”	”	”	”	$\frac{2}{28}$	41	2,870	2,905		
”	*p*	”	”	”	”	”	42	2,940			
”	T_1	”	”	”	not	$\frac{1}{27}$	140	3,780	3,780		
”	T_2	”	”	”	”						
”	*c*	”	”	72	”	$\frac{1}{29}$	230	6,670	7,250	6,442	7,881
”	*d*	”	”	”	”	”	270	7,830			
”	*g*	”	”	”	$\frac{1}{3}$	$\frac{1}{27}$	78	6,318	5,467		
”	*h*	”	”	”	”	”	57	4,617			
”	*k*	”	”	”	$\frac{1}{5}$	$\frac{1}{28}$	41	5,740	6,160		
”	*l*	”	”	”	”	”	47	6.580			
”	*o*	”	”	”	”	$\frac{2}{28}$	113	7,910	8,890		
”	*p*	”	”	”	”	”	141	9,870			
”	T_1	”	”	”	not	$\frac{1}{27}$	431	11,637	11,637		
”	T_2	”	”	”	”						

Table S—*continued.*

Date.	Plate.	Age of water.	Temperature of incubation.	Time incubated.	Diluted or not.	Number of drops.	Colonies on plate.	Calculated number of bacteria per 1 c.c.	Averages.	Total average.	
				hours.		c.c.					
Dec. 28, 1893	*c*	$\frac{1}{2}$ hour at 8—10° C.	18—20° C.	96	not	$\frac{1}{29}$	312	9,048	9,642	10,182	11,434
,,	*d*	,,	,,	,,	,,	,,	353	10,237			
,,	*g*	,,	,,	,,	$\frac{1}{3}$	$\frac{1}{27}$	78	6,318	6,763		
,,	*h*	,,	,,	,,	,,	,,	89	7,209			
,,	*k*	,,	,,	,,	$\frac{1}{5}$	$\frac{1}{28}$	72	10,080	9,940		
,,	*l*	,,	,,	,,	,,	,,	70	9,800			
,,	*o*	,,	,,	,,	,,	$\frac{2}{28}$	201	14,070	14,385		
,,	*p*	,,	,,	,,	,,	,,	210	14,700			
,,	T_1	,,	,,	,,	not	$\frac{1}{27}$	609	16,443	16,443		
,,	T_2	,,	,,	,,	,,						
,,	*c*	,,	,,	120	,,	$\frac{1}{29}$	Liquid	..	12,006	13,072	13,746
,,	*d*	,,	,,	,,	,,	,,	414	12,006			
,,	*g*	,,	,,	,,	$\frac{1}{3}$	$\frac{1}{27}$	Liquid	..	10,044		
,,	*h*	,,	,,	,,	,,	,,	124	10,044			
,,	*k*	,,	,,	,,	$\frac{1}{5}$	$\frac{1}{28}$	98	13,720	13,370		
,,	*l*	,,	,,	,,	,,	,,	93	13,020			
,,	*o*	,,	,,	,,	,,	$\frac{2}{28}$	222	15,540	16,870		
,,	*p*	,,	,,	,,	,,	,,	260	18,200			
,,	T_1	,,	,,	,,	not	$\frac{1}{27}$	609	16,443	16,443		
,,	T_3	,,	,,	,,	,,						
,,	*d*	,,	,,	144	,,	$\frac{1}{29}$	Liquid	..	12,006	14,804	15,132
,,	*h*	,,	,,	,,	$\frac{1}{3}$	$\frac{1}{27}$	130	10,530	10,530		
,,	*k*	,,	,,	,,	$\frac{1}{5}$	$\frac{1}{28}$	137	19,180	16,730		
,,	*l*	,,	,,	,,	,,	,,	102	14,280			
,,	*o*	,,	,,	,,	,,	$\frac{2}{28}$	225	15,750	19,950		
,,	*p*	,,	,,	,,	,,	,,	345	24,150			
,,	T_1	,,	,,	,,	not	$\frac{1}{27}$	Liquid	..	16,443		
,,	T_2	,,	,,	,,	,,						
,,	*h*	,,	,,	168	$\frac{1}{3}$	,,	130	10,530	10,530	16,331	16,331
,,	*k*	,,	,,	,,	$\frac{1}{5}$	$\frac{1}{28}$	160	22,400	18,340		
,,	*l*	,,	,,	,,	,,	,,	102	14,280			
,,	*o*	,,	,,	,,	,,	$\frac{2}{28}$	225	15,750	20,125		
,,	*p*	,,	,,	,,	,,	,,	350	24,500			

In the following plates I have plotted the curves obtained from the means of the foregoing illustrative series of analyses. They bring out very clearly the differences in numbers referred to, and I am strongly of opinion that much valuable information would result from a systematic series of monthly analyses of the Thames water conducted along these lines.

I by no means pretend that the numbers themselves are of absolute value, any more than are those obtained by the ordinary methods of counting averages; but I do think that the selected cases suggest possible lines of departure for the systematic bacteriological analysis of such a river as the Thames, if a sufficient number of other data were taken in at the same time. These data should include at least the following: (1) the temperature of the river, (2) the amount of sunshine, (3) the organic analysis of the water, (4) the rainfall.

I am perfectly alive to the incompleteness of the above analyses in these respects, and they are only intended to show what I think should be done by a competent staff of assistants, if any attempt is made at a thorough investigation of the bacteriology of the Thames and the same applies to any other water.

The particular object of the above analyses was to test the view that the actual number of bacteria present in winter is less than that in summer, and they strongly confirm that; and if we remember that this was so in 1893 in spite of (1) the river being lower in August, and therefore more concentrated as a food liquid, (2) the temperature being higher, and therefore more favourable to bacterial growth, it seems at least highly probable that the diminution in the bacteria is largely due to the increased insolation.

Nor is this all (though I defer the fuller consideration of this point) that my analyses suggest. I find very distinct evidence that the bacteria in the summer water are many of them *enfeebled* forms, suggesting a distinct inhibition or weakening of their powers of growth. In some cases it is certain that forms obtained in August, and which afterwards turned out to be identical with forms found in the winter, at first grew so feebly that their characters on the plates led one to put them down as distinct species or varieties.

I have given some experimental evidence bearing on this, and going to prove that it is due to the action of light on these forms. The matter is a very complex one, and I must refrain from further discussion of it until all the forms isolated during the year are worked out; but it is worth while, I think, to draw the attention of investigators to the matter. In one or two cases, at least, there is no question that exposure to light *does* so affect the germination and growth of the bacteria, that the resulting colonies depart widely from the normal in many of their characters.

I have in hand a large number of experimental results obtained

from the detailed investigation of a single species and the measurement of the growth of a single filament (watched continuously under the microscope) under different conditions of exposure; they have been incidentally referred to in my lecture at the Royal Institution, in May, 1894, and I shall hope to bring them before the Royal Society shortly. They prove still more conclusively at least the main point, that exposure to sunlight does materially affect the rate and manner of growth, &c., of a bacterium rodlet or filament, as well as the germinal power of the spores.

As regards further details on the action of light on the spores and bacilli, I may refer the Committee to my memoir, now in the hands of the Royal Society, an abstract of which appeared in the 'Proceedings,' vol. 54, p. 472.

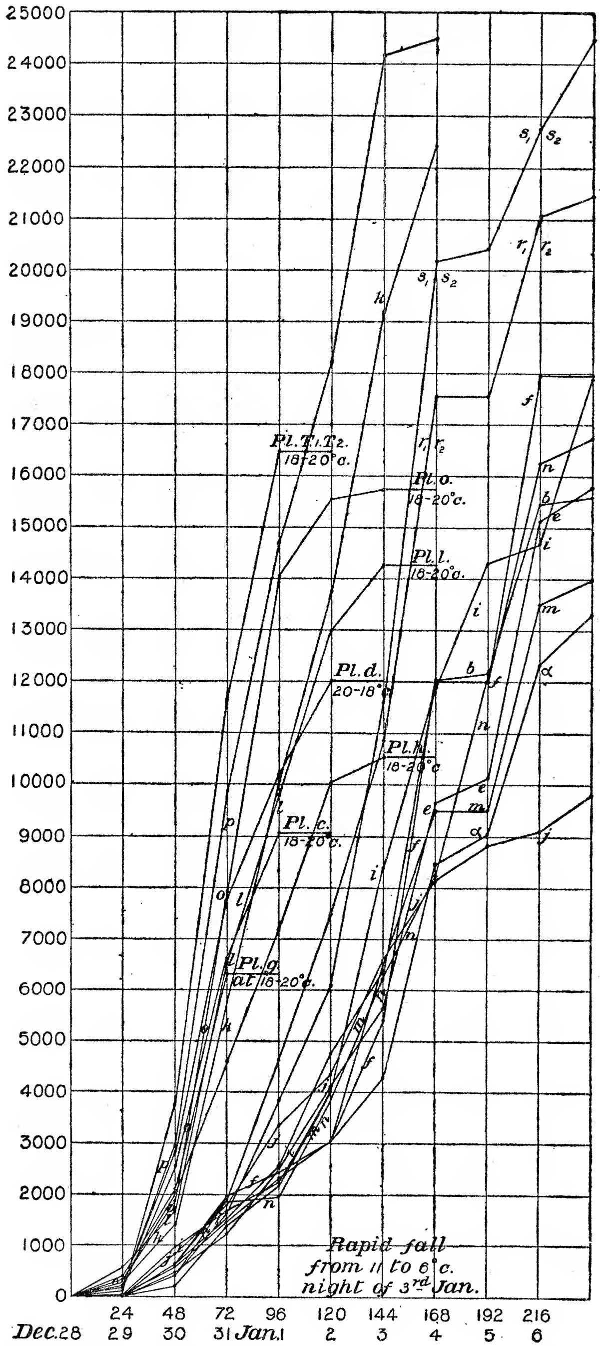


DIAGRAM IV.—Curves of Table S, showing total number of bacteria at 10—12° and 18—20° C. in December. Abscissæ = hours of incubation; ordinates = numbers of bacteria per 1 c.c.

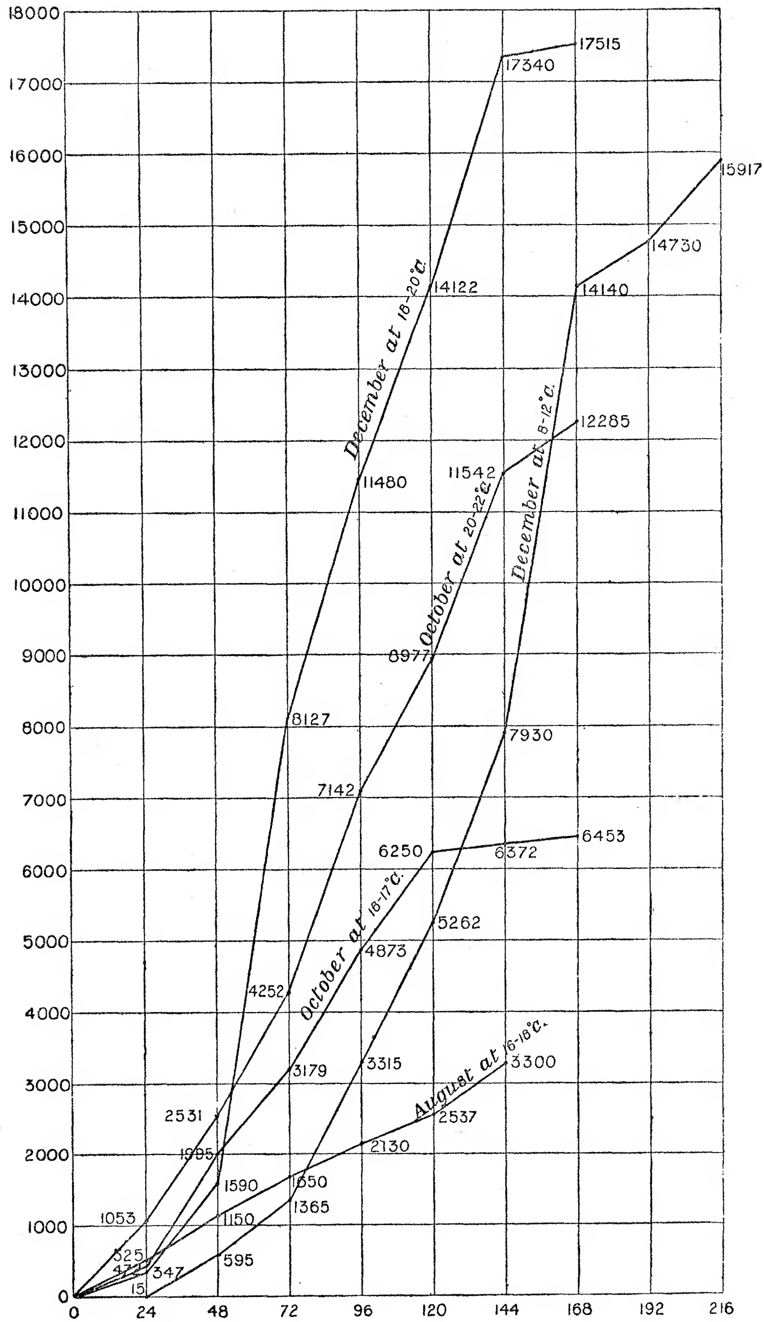


DIAGRAM V.—Means of the foregoing curves, showing average numbers of bacteria for August, October, and December.

Part II.

"The Behaviour of the Typhoid Bacillus and of the *Bacillus Coli Communis* in Potable Water." By Percy Frankland, Ph.D., B.Sc. (Lond.), F.R.S., Professor of Chemistry in Mason College, Birmingham, assisted by J. R. Appleyard, F.C.S.

It has already been pointed out in the previous Reports that the only two zymotic diseases which have been conclusively proved to be communicable, and frequently communicated by drinking water, are Asiatic cholera and typhoid fever. The behaviour in water of the particular micro-organisms which are almost universally credited with the power of exciting these specific maladies is obviously, therefore, one of the most interesting and important questions in the whole domain of the hygiene of water supply.

Inasmuch as Asiatic cholera is, fortunately, only an occasional visitor of these islands, or, indeed, of the continent of Europe, the investigation of this question with regard to this disease is certainly of less immediate consequence than is its investigation with regard to typhoid fever, which we have always with us, and which claims such a large number of victims annually from amongst our population.

In the present Report I have, therefore, endeavoured on the one hand to summarise briefly what has already been done by others towards the elucidation of this subject of the behaviour of the typhoid bacillus in water, whilst on the other hand I have recorded those experiments which I have myself conducted with a view to extending the knowledge of this matter in general, and in respect to the conditions of water-supply pertaining to this country in particular.

Our information concerning the behaviour of the typhoid bacillus in water is of essentially two different kinds; firstly, this bacillus has on a number of occasions been discovered with more or less certainty in waters which were actually being used for domestic purposes, and to which it had therefore gained access unintentionally and in the natural course of events; secondly, the bacillus has been purposely introduced into various waters in which its subsequent fate has then been traced by experimental observation. It will be convenient to consider these two different kinds of information separately.

1. *Discovery of the Typhoid Bacillus in Natural Waters.*

Inasmuch as the communicability of typhoid fever by drinking water has been long recognised as a cardinal principle of modern hygiene, it was only natural that the discovery of the specific micro-organism of this disease by Eberth should have been soon followed by strenuous efforts to discover the Eberth-bacillus in potable water; it was not until about six years afterwards, however, that successful attempts in this direction were announced.

The first investigator who claimed to have discovered Eberth-Gaffky's bacillus in water was Moers ("Die Brunnen der Stadt Mühlheim a. Rhein vom bakteriologischem Standpunkte aus betrachtet," 'Ergänzungsh. zum Centralblatt f. allgem. Gesundheitspflege,' vol. ii, 1886, p. 144), who isolated the bacillus from a contaminated well supplying drinking water to a number of people amongst whom many cases of typhoid fever had occurred.

This discovery was soon followed by a similar announcement from Michael ("Typhusbacillen im Trinkwasser," 'Fortschritte d. Medicin,' vol. iv, 1886, p. 353), in Dresden, who claimed to have isolated the bacillus from a well-water which was suspected of being the source of an outbreak of typhoid which declared itself at the end of the year 1885.

Dreyfus-Brisac and Widal ("Epidémie de Famille de Fièvre typhoïde," 'Gaz. hebdom.,' 1886, No. 45) again detected the bacilli in the polluted water of a well at Ménilmontant, where typhoid fever had been prevalent for some months.

The typhoid bacillus has repeatedly been found in the water of the river Seine, thus Chantemesse and Widal ('Gaz. hebdom. de Méd. et de Chirurg.,' 1887, pp. 146—150; 'Centralbl. f. Bakteriol.,' vol. i, 1887, p. 682) discovered the bacillus no less than three times in this water during an outbreak of typhoid in Paris. Thoinot ('La Semaine Médicale,' 1887, No. 14, p. 135; 'Centralbl. f. Bakteriol.,' vol. ii, 1887, p. 39) also isolated typhoid bacilli from the Seine at Ivry, at a distance of but little more than twenty yards from the point where the water is abstracted for the occasional supply of Paris with drinking water. Again, Loir ("Recherche du Bacille typhique dans les Eaux d'Alimentation de la Ville de Paris," 'Annales de l'Institut Pasteur,' vol. i, p. 488) detected the typhoid bacillus in Seine water which was actually being distributed to a portion of Paris during the summer of 1887, owing to the scarcity of the Vanne water, which yields the supply under ordinary circumstances. Vincent ("Présence du Bacille typhique dans l'Eau de Seine pendant le Mois de Juillet, 1890," 'Annales de l'Institut Pasteur,' vol. iv, p. 772) again found the typhoid bacillus in the Seine water which was being similarly supplied to Paris during the summer of 1890.

Beumer ("Zur Aetiologie d. Typhus abdominalis," 'Deutsche medicinische Wochenschrift,' 1887, No. 28) was able to detect the typhoid bacillus in a well-water used for drinking purposes in a country place near Greifswald, where an outbreak of typhoid had occurred.

A similar discovery of the bacillus was made by Brouardel and Chantemesse ("Enquête sur les Causes de l'Epidémie de Fièvre typhoïde qui a régné à Clermont-Ferrand," 'Annales d'Hygiène publique et de Médecine légale,' vol. xvii, 1887, pp. 385—403; also 'Revue d'Hygiène,' vol. ix, p. 368) in the course of an investigation of an epidemic of typhoid which prevailed at Clermont-Ferrand, and in which neighbouring places using the same water supply were also involved.

Finkelnburg ("Ueber einen Befund von Typhusbacillen im Brunnenwasser," 'Centralbl. f. Bakteriol.,' vol. ix, p. 301) states that he isolated the typhoid bacillus from a well which had in all probability been contaminated with typhoid dejecta.

Henrijean ("Contribution à l'Etude du Rôle étiologique de l'Eau potable dans les Epidémies de Typhus," 'Annales de Micrographie,' vol. ii, p. 401) found typhoid bacilli in the drinking water of a Belgian village during an epidemic of typhoid fever.

Kamen ("Zum Nachweise der Typhusbacillen im Trinkwasser," 'Centralbl. f. Bakteriol.,' vol. xi, p. 32) detected typhoid bacilli in water supplying a Russian military garrison, amongst whom typhoid fever had broken out.

Péré ("Contribution à l'Etude des Eaux d'Alger," 'Annales de l'Institut Pasteur,' vol. v, p. 79) states that he was able to isolate the typhoid bacillus from drinking water in Algiers, where typhoid is endemic, occurring every year during the months of August, September, and October.

Martin states ("Présence du Bacille typhique dans les Eaux d'Alimentation de la Ville de Bordeaux," 'Revue sanit. de la Province,' 1891, No. 181, p. 93; 'Centralbl. f. Bakteriol, vol. xi, 1892, p. 413) that the typhoid bacillus was found by Ponchet in the public water supply of Bordeaux during an outbreak of typhoid in that city.

Fodor ("Die Beziehungen des Typhus zum Trinkwasser," 'Centralbl. f. Bakteriol.,' vol. xi, 1892, p. 121), in a paper read at the International Hygienic Congress held in London in 1891, describes an outbreak of typhoid fever at Budapest, during which he succeeded in detecting the typhoid bacillus no less than five times in the public water-supply. It was afterwards ascertained that the waste water from a laundry attached to the hospital gained direct access to the principal water main in the town.

Kowalski ("Ueber bakteriologische Wasseruntersuchungen,"

'Wiener klinische Wochenschrift,' 1888; 'Centralbl. f. Bakteriol.' vol. iv, 1888, p. 467) states that out of 2000 samples of water which he had examined bacteriologically, there were only five in which he was able to detect typhoid bacilli.

From the above it will be seen that many investigators, since the year 1886, claim to have discovered the typhoid bacillus in potable waters, but, in the majority of cases, these discoveries, especially the earlier ones, must be accepted with considerable reserve, as, until recently, it was customary to rely for the identification of the typhoid bacillus on altogether insufficient data, as it only gradually became understood that there are several other forms presenting the closest points of resemblance in their morphological characters, both micro and macroscopic, to the typhoid bacillus, with which they are not unfrequently associated, and, moreover, some of these simulatory forms are of very frequent occurrence in natural waters. Indeed, even at the present day, the identification of particular forms or "species" of bacteria is in a transitional and highly unsatisfactory state, as it is daily becoming clearer that the characters, both morphological and physiological, of one and the same micro-organism are often liable to the profoundest modifications through changes of environment and other causes, whilst there are almost daily being discovered in nature new forms which differ only from already well-known forms or "species" in what appear to be the most minute, trifling, and insignificant particulars.

Under these circumstances, it is practically certain that some of the bacilli discovered in water, and believed to have been typhoid bacilli, must, in reality, have been only forms closely simulating the more striking characters of the typhoid bacillus. On the other hand, it is equally certain that a great many waters which have been submitted to examination for typhoid bacilli may have contained them without their being discovered, for, as will be pointed out later, the ordinary method pursued in the bacteriological examination of water, in which a few drops, or at most a cubic centimetre or two, of the water is submitted to plate cultivation, can only, under the most exceptional circumstances, lead to the detection of typhoid bacilli.

Thus, whilst there is considerable evidence that the typhoid bacilli have been found on a number of occasions in waters which had been convicted of distributing typhoid amongst their consumers, the failure to discover these bacilli in other waters equally guilty need excite no surprise when the very imperfect methods of examination which are commonly employed for their discovery are taken into consideration.

In connection with the above discoveries of typhoid bacilli in potable water, I will only at this stage further remark that, in by far the majority of cases, the waters accused of containing these bacilli

were well waters, and, as is well known, it is just this kind of water which has most frequently been conclusively convicted of distributing typhoid fever.

The above discoveries of the typhoid bacillus in natural waters have, in almost all cases, been made not by means of the ordinary method of plate cultivation, which affords little or no chance of a few typhoid bacilli being discovered amongst a host of common water bacteria, the colonies of which generally grow with great rapidity and not unfrequently cause rapid liquefaction of the gelatine, but by special methods of treatment which have been devised to oppose the proliferation of the water bacteria whilst not materially interfering with the growth and multiplication of the typhoid bacilli, the latter thus acquiring a large numerical preponderance, if not entirely excluding the other forms present in the water.

Unfortunately, these conditions which foster the growth of the typhoid bacillus to the exclusion of the ordinary water bacteria are equally propitious to other microbes which are invariably associated with the typhoid bacillus, and which, in fact, resemble it so closely, especially in morphological characters, that they may easily be mistaken for the typhoid bacillus, and it is this circumstance which causes so much doubt to attach more especially to the earlier of those alleged discoveries of the typhoid bacillus in potable water which are recorded above.

The particular micro-organism, which is especially liable to cause confusion in this respect, is the so-called *Bacillus coli communis*. This organism was described by Escherich, and is found regularly in the human intestinal tract and fæces, as well as in the excreta of other animals. It is regarded as identical with the *Bacillus neapolitanus* (Emmerich), and the "Fæces bacillus" described by Weisser, whilst by recent experiments I have shown that it is closely allied to, if not identical with, the *Bacillus ethacetosuccinicus* previously described by me. In all cases, therefore, in which water is supposed to have been infected with the dejecta of typhoid patients, the *B. coli communis* may be expected also to be present. In order, therefore, to ascertain definitely whether the typhoid bacillus is present in any given water, care must be taken that the *B. coli communis* is not mistaken for the former, and to guard against this it would be a desideratum to have some method which would, whilst revealing the presence of the typhoid bacillus, effectually eliminate or separate out its almost constant attendant, the *B. coli communis*. Unfortunately, this is a consummation which has not yet been realised in fact, for not only is the vitality of the *B. coli communis* in water, as will be shown below, superior to that exhibited by the typhoid bacillus, but in all attempts which have so far been made to suppress the vitality of other organisms, and yet permit of the

development of the latter, the *B. coli communis* has shown itself to be possessed of greater powers of resistance than the typhoid bacillus itself. Hence, although the addition of various chemical substances in suitable proportions may effectually destroy or retard the growth of other organisms, yet the *B. coli communis* survives and remains present along with the typhoid bacillus; indeed, in many cases it has been shown that such additions have actually destroyed the typhoid bacillus, and left the *B. coli communis* alone master of the field. It is true that, growing in artificial cultures side by side, there are certain differences observable between these two organisms, for the *B. coli communis* grows more luxuriantly in the various culture-media employed than does the typhoid bacillus, or to use the expressive language of a French observer, the *B. coli communis* grows as though it were *well*, and the typhoid bacillus as though it were *ill;* but yet on the gelatine plates of each there are nearly always colonies which are indistinguishable from those of the other, whilst even in the potato-cultures, which used to be regarded as the crucial test for the typhoid bacillus, the *B. coli communis* may and does exhibit, under certain conditions, growths which resemble in every respect those produced by the typhoid bacillus. In two media, however, as has been pointed out by Dunbar ("Ueber den Typhusbacillus und den Bacillus Coli Communis," 'Zeitsch f. Hygiene,' 1892, 491), a marked difference is found in the behaviour of these two organisms. Thus, when inoculated into sterile milk, the typhoid bacillus renders the liquid slightly acid, but *never* causes its coagulation; the *B. coli communis*, on the other hand, at the temperature of the body coagulates the milk in from 24—48 hours, rendering it at the same time strongly acid. Again, when grown in sterile fluid meat extract or broth, the *B. coli communis* at 37° C. produces, in the course of from 3—12 hours, a quantity of gas (consisting of hydrogen and carbonic anhydride), whilst no formation of gas has, under the same conditions, ever been observed in the case of the typhoid bacillus.

This latter mode of distinguishing between the *B. coli communis* and the typhoid bacillus I have reduced to the following extremely simple and handy form suitable for their rapid differentiation:—The organism under investigation is inoculated into a test-tube containing *ordinary gelatine peptone* in a melted state, the latter is shaken to distribute the bacilli throughout the liquid, which is then allowed to solidify and maintained at the ordinary temperature (18—20° C.). The tube, if it contains the *B. coli communis*, will invariably, after 12—48 hours, exhibit numerous conspicuous gas bubbles distributed through the solid medium, whilst no such bubbles make their appearance in similar tubes containing the typhoid bacillus. The test possibly depends upon the meat extract containing sufficient dextrose (derived from the *post-mortem* transformation of the glycogen

in the blood) for a visible fermentation by the *B. coli communis* to take place. The bubbles of gas are certainly independent of any ingredients present in either the gelatine or in the peptone, for I have found them to form also in agar-agar-peptone, and also in meat-extract gelatine to which no peptone had been added.* The great convenience of the test depends upon its involving only the use of a medium which must invariably be at hand at all times in every bacteriological laboratory, and also on its dispensing with the use of an incubating temperature, whilst it has the further advantage over Dunbar's original broth-bubble test that the bubbles of gas being fixed in the solid gelatine, the tubes can be examined at leisure even days or weeks after inoculation, whilst with the broth-bubble test, if the tubes are not examined at the right time, the fermentation may have ceased; besides, in the broth, of course, the bubbles are not nearly so conspicuous. Extensive use has been made of this method during the present investigation, and for rapidly and certainly distinguishing between the typhoid bacillus and the *B. coli communis* I have found it unequalled; on the other hand, it must be borne in mind that it does not serve to distinguish between the *B. coli communis* and many other fermenting organisms.

A further but less certain distinction which should also be employed for differentiating between the typhoid bacillus and the *B. coli communis* is the so-called *indol-reaction*. This test is best applied in the following manner, as recommended by Kitasato :—

To 10 c.c. of the culture in ordinary alkaline peptone-broth of the organism under examination, and which has been growing for twenty-four hours in the incubator, add 1 c.c. of a solution of potassium or sodium nitrite (containing 0·02 gram in 100 c.c.) and then a few drops of concentrated sulphuric acid. *If indol is present, a rose to deep-red coloration is produced,* depending on the interaction of nitrous acid with indol to form nitrosoindol nitrate which is of red colour. On applying this test to the *B. coli communis* an indol-reaction should be obtained, whilst the typhoid bacillus gives invariably a negative result. In practice I have found it advisable not to apply the indol-test until the broth-culture has been forty-eight hours in the incubator.

Although the *B. coli communis* is generally supposed to give the indol-reaction, this character would appear not to be so constant as is commonly imagined. In my own experiments I have known *one and the same culture-series* of the *B. coli communis* not to give the indol-reaction at one time, and yet subsequently to become possessed of this power, although I have not been able to determine the cause which leads to the loss of the indol-producing capacity. Thinking

* Dunbar found also that no bubbles were formed in a solution of peptone without meat extract.

that it might possibly be due to the growth of the bacillus having become enfeebled, I tried growing it under unfavourable conditions, viz., in phenol-broth, in which I left the bacillus for months without transplanting, but even by this severe treatment I found no diminution in the indol-producing power. The absence of indol-production by the *B. coli communis* has also been noticed by Dunbar, who in his exhaustive memoir (*loc. cit.*) compares the behaviour of a culture of the typhoid bacillus with a culture of the *B. coli communis*, and found that neither bacilli gave the indol-reaction. It is obvious, therefore, that indol production is not a necessary attribute of the *B. coli communis*, and that too much reliance must not be placed on it as a means of distinguishing between the typhoid bacillus and the *B. coli communis*.

As convenient, for purposes of reference, I have collected in the following two tables the principal characters of these two bacilli :—

Typhoid Bacillus.

(*Bacillus typhi abdominalis.*)

Authority.—Eberth, 'Virchow's Archiv,' vol. 81, 1880; also *ibid.*, vol., 83, 1881. Gaffky, "Zur Aetiologie des Abdominaltyphus," 'Mittheilungen a. d. Kaiserlichen Gesundheitsamte,' vol. 2, 1884, p. 372.

Where Found.—In the blood, urine, fæces, as well as in the organs of typhoid patients. Found by numerous investigators in water.

Microscopic Appearance.—A short, plump bacillus about three times as long as broad, with rounded ends. It occurs in the tissues usually singly, but in artificial cultures it grows frequently into long threads. It is very motile and is provided with numerous cilia, which are attached to both the sides and ends of the bacillus. To stain the cilia add 22 drops of caustic soda to 16 c.c. of the mordant (Loeffler). It is not stained by Gram's method, and stains less readily with aqueous aniline solutions than most bacteria. Günther recommends heating the cover-glass, after the dye has been poured on it, for a few seconds until it begins to steam, and then washing off the stain as usual. It does not form spores.

Cultures: Gelatine Plates.—The colonies on the surface form large spreading greyish-white iridescent expansions with jagged and irregular edge. Under a low power they exhibit a brownish shimmer and a characteristic woven structure. The depth colonies are darker, with regular edge, and are covered with delicate irregular lines. No liquefaction takes place.

Gelatine Tubes.—Grows chiefly on the surface, producing a delicate greyish-white iridescent expansion with irregular edge.

Agar-agar.—Forms a greyish-white moist expansion.

Potatoes.—Produces an almost invisible greyish-white growth after forty-eight hours, but on touching the moist-looking surface with the needle a tough resistant pellicle is found. On some potatoes, however, its growth is more apparent, so that the above is not the only appearance to which it may give rise.

Blood Serum.—Produces a milk-white expansion restricted to the path of the needle.

Broth.—Renders it turbid.

Milk.—Grows abundantly, rendering it slightly acid. No coagulation takes place.

Remarks.—It grows best at about 37° C. Kitasato states that it produces no indol reaction. It produces sulphuretted hydrogen in iron-gelatine, the needle-track after from five to six days becoming intensely black in colour. In iron-agar, at from 33° to 34° C., this black colour appears at the end of 24 hours (Fromme). It produces sulphuretted hydrogen in broth with or without peptone; comparative tests made with the *B. coli communis* revealed no difference either in the degree of the reaction (as shown by the lead-paper test) or in the rapidity with which it took place in the case of the two organisms. The typhoid bacillus never produces gas in any artificial media. It is destroyed when heated for ten minutes at 60° C.

Injection into the aural vein of rabbits causes death in 24—28 hours (Fraenkel and Simmonds), guinea-pigs into which the cultures are introduced by the mouth, as described for cholera, are also killed (Seitz). Opinion is, however, still divided as to whether death is due to mere intoxication by the bacterial products present in the cultures, or to actual multiplication of the bacillus within the animal. In this connection, see Petruschky ('Zeitsch. f. Hygiene,' vol. 12, 1892, p. 269).

Bacillus Coli Communis.

Authority.—Escherich, 'Fortschritte der Medicin,' vol. 3, 1885, Nos. 16 and 17. Also Dunbar, "Ueber den Typhus-bacillus und den Bacillus Coli-communis," 'Zeitschrift f. Hygiene,' vol. 12, 1892, p. 485. Also Luksch, "Zur Differenzialdiagnose des Bacillus typhi abdominalis (Eberth) und des Bacterium Coli-commune (Escherich)," 'Centralblatt f. Bakteriologie,' vol. 12, 1892, p. 427.

Where Found—In the intestinal tract of man and animals. Found often in water by numerous investigators, and frequently mistaken for the typhoid bacillus.

Microscopic Appearance.—The typical form is a short bacillus 0·4 μ broad and 2 to 3 μ long; it is, however, very variable, oval individuals and forms resembling cocci being also found. It exists chiefly as a double bacillus arranged in groups. It is slightly motile, and is provided with 1 to 3 cilia, whilst the typhoid bacillus has 8 to

12 cilia (Luksch). Nicolle and Morax mention that the *coli* bacillus has invariably fewer cilia than the typhoid, that whereas the former rarely possesses more than six, the latter usually exhibits ten to twelve, whilst the cilia of the former are also far more fragile ('Annales de l'Institut Pasteur,' vol. 12, 1893, p. 561). It does not form spores.

Cultures: Gelatine Plates.—Forms round, and very often oval, smooth-rimmed granular colonies in the depth, which later become yellowish-brown in colour. On the surface it forms flat, irregular, pale white expansions, which under a low power exhibit a furrowed appearance due to the unequal thickness of the colony in its different parts. The colony also presents a distinctly wavy lineal structure parallel to the periphery. No liquefaction ensues.

Gelatine Tubes.—Grows somwhat abundantly in the depth, producing small white pin-head colonies, whilst on the surface it forms an expansion resembling the growth on gelatine plates.

Agar-agar.—Grows abundantly on the surface, producing a dirty-white, faintly shining expansion.

Blood Serum.—Forms a milk-white expansion.

Potatoes.—Produces a slimy yellow expansion on some potatoes, on others grey-white, whilst in some cases it resembles the typhoid bacillus in being hardly visible.

Broth.—Renders it turbid.

Milk.—Renders it acid, and at 37° C. coagulates it in from twenty-four to forty-eight hours.

Remarks.—Both cultures of twenty-four hours' age generally exhibit considerable evolution of gas; ordinary gelatine or agar stab-cultures also generally exhibit bubbles of gas in the solid medium. Such bubbles can invariably be obtained by inoculating into ordinary melted gelatine, which is afterwards allowed to solidify (Percy Frankland). The addition of dextrose to the gelatine is quite unnecessary for this purpose. Exhibits indol reaction after twenty-four to forty-eight hours' culture in peptone broth.

Is capable of exhibiting very different degrees of pathogeneity according to its origin, cultures made from diseased tissues in which it is present on being intraperitoneally inoculated into rabbits cause peritonitis, and the bacilli are found in pure culture in the heart's blood. (Alex. Fraenkel, 'Wiener klin. Wochenschr.,' 1891, Nos. 13—15.)

2. *Behaviour of Typhoid Bacilli experimentally introduced into Potable Water.*

The second kind of information concerning the behaviour of the typhoid bacillus in water has, as already mentioned, been gained by

direct experiment, *i.e.*, by purposely introducing the bacillus into water, and in this manner the conditions which are favourable and unfavourable to the vitality in water of the typhoid bacillus can, of course, be much more readily ascertained than by the study of such chance cases as those already enumerated above, in which the bacilli had, in the natural course of events, gained access to water.

I have below expressed in a tabular form the principal results of the numerous investigators who have already availed themselves of this method of experimenting on the behaviour of the typhoid bacillus in water.

From this table it will be seen that different observers ascribe very different degrees of vitality to the typhoid bacillus in water, nor is this to be wondered at when it is remembered that the typhoid bacilli introduced into the waters may have been possessed of very different degrees of initial vitality according to their age and previous history; whilst, secondly, the waters experimented with were, of course, not the same; thirdly, the amount of food-material introduced into the water along with the bacilli must have been subject to the very greatest variations, and again the temperatures and other conditions under which the infected waters were preserved were equally variable.

Thus, taking the experiments made with distilled water, in which, therefore, there is the most chance of the water having been of uniform quality, Braem found the introduced typhoid bacilli still alive after 188 days, whilst the longest duration of vitality in this medium observed by Hochstetter was five days, Meade Bolton, Slater, Straus and Dubarry, and Wolffhügel and Riedel giving periods intermediate between these two wide extremes. These discrepancies in the case of distilled water are doubtless to be accounted for partly by the difference in initial vitality possessed by the different typhoid bacilli employed and partly by the difference in the amount of culture-material imported into the distilled water along with the bacilli themselves, whilst the actual numbers in which the bacilli were introduced may also greatly influence the degree of longevity observed.

This wide divergence in the results obtained by previous observers would alone call for a reinvestigation of this subject with a view to ascertaining the longevity of the typhoid bacillus in definite types of British potable water, and taking more into consideration the exact chemical composition of the waters experimented with.

There is, however, another point arising out of the results arrived at by previous investigators which still more urgently demands reinvestigation with a view to its confirmation, qualification, or direct contradiction, and this is the relatively far greater longevity of the typhoid bacillus in sterilised than in unsterilised potable water,

which has been affirmed more especially by Kraus and subsequently by Karlinski. This point is obviously of the very highest importance from a practical hygienic point of view, as it is with unsterile potable water that we are in practice alone concerned, and the duration of vitality ascribed to the typhoid bacillus in such water by both these observers is of very limited extent—not more than seven days.

The experiments of Kraus are so striking, and have attracted so much attention, that I will give them in more detail in the following table :—

Typhoid Bacillus.

Description of Water.	Number of Days after Inoculation when Examined.							
	1.	2.	3.	5.	7.	9.	20.	150.
	Number of Typhoid Bacilli found in 1 c.c. of Water.							
(1) Munich water supply (Mangfall)	57,960	50,400	15,680	9,000	0	0	0	0
(2) Well-water, Munich......	57,000	50,840	32,643	8,900	0	0	0	0
(3) ,, ,,	56,000	35,910	10,010	7,060	0	0	0	0
	Number of Water Bacteria found in 1 c.c. of Water.							
(1) Munich water supply (Mangfall)	0	0	0	80	288,000	400,000	970,000	1,080
(2) Well-water, Munich......	0	0	490	lost	300,000	427,000	innumerable	1,980
(3) ,, ,,	0	0	280	500	256,000	lost	456,000	1,050

These results indicate, therefore, that, on introducing the typhoid bacilli into the potable waters in question, which were almost naturally sterile, the typhoid bacilli promptly disappeared as soon as the water bacteria had undergone extensive multiplication, which had taken place in each of the three experiments by the seventh day after the importation of the typhoid bacilli.

These interesting experiments cannot, however, by the light of our present knowledge, be accepted without criticism, for there cannot be the slightest doubt that, when only the ordinary method of plate-cultivation is employed in such investigations on unsterilised water, the typhoid bacilli will be generally overlooked unless they are present in large numbers. Again, the attempts which have been made, both by Kraus and other experimenters, to count the typhoid colonies on plates containing such mixtures of colonies, and the numerical estimates given of the typhoid bacilli in such unsterilised waters, must be wholly illusory, for the number of typhoid colonies which develope what may be called a typical appearance (*i.e.*, one which enables them to be readily recognised with reasonable certainty) depends on a variety of different circumstances, amongst which may be mentioned the age of the plate, the extent to which

the colonies are crowded together on the plate, very probably, also, the nature of the other colonies on the plate, and certainly the degree of vitality possessed by the typhoid bacilli themselves. Thus, if the numerical estimate of the typhoid colonies on a plate is made by counting as such the characteristic surface expansion colonies only, the result must be entirely fallacious, as nothing is, in my experience, commoner than to find only a vanishing proportion of the total number of typhoid colonies, even on a pure plate, giving rise to these surface-expansions at all.

In looking for typhoid bacilli in such artificially-infected unsterile waters it is, in fact, necessary to employ special methods for their detection similar to those which, as already pointed out, had to be devised for the examination of natural waters for the typhoid bacillus, and it is only when such special methods have been employed with a negative result that the conclusion can be legitimately drawn that the typhoid bacillus is not present in the living state in the particular volume of water operated on.

In the present investigation, the uniform practice has been made of examining all unsterile waters by means of Parietti's method of phenol broth-culture (see description below) in order to ascertain the presence or absence of the typhoid bacillus or of the *B. coli communis*.

Method of Detecting the Typhoid Bacillus and Bacillus Coli Communis *in Unsterile Waters.*

It will not be necessary to describe the various methods which have been devised for discovering the presence of typhoid bacilli in water, but it will be sufficient to point out that these are nearly all based upon the fact that the typhoid bacillus is, in comparison with most bacteria, but little affected by small doses of either phenol or dilute acids, so that, by adding suitable quantities either of phenol alone or of phenol in conjunction with acid to the culture-media, the growth and multiplication of the typhoid bacillus is not materially interfered with, whilst the proliferation of most, if not of all, the water-bacteria is suppressed.

Of these various methods, the one which I selected for the purposes of this investigation was that devised by Parietti ("Metodo di ricerca del Bacillo del tifo nelle aque potabili," 'Rivista d' Igiene e Sanità pubblica,' 1890). This method, which consists in adding phenol along with hydrochloric acid in certain proportions to neutral broth is carried out as follows:—

A series of test-tubes containing 10 c.c. of neutral broth, each receive from 3 to 9 drops of a solution having the following composition:—

Phenol	5	grams.
Hydrochloric acid (pure)	4	,,
Distilled water................	100	,,

(In practice, I generally employ some tubes to which 3 drops (= 0·25 c.c.) and others to which 5 drops (= 0·4 c.c.) of this solution have been added.)

To the tubes thus treated, from 1 drop to several cubic centimetres of the water under investigation are added, and, after thoroughly mixing the contents, the tubes are placed in the incubator at 37° C. As soon as the tubes become turbid (which in the initial presence of many typhoid bacilli will occur already in twenty-four hours, but if only few are present, may be postponed for forty-eight, seventy-two, or even more hours) they are submitted to ordinary plate cultivation in three dilutions, the second and third dilutions only being actually poured on to plates or into Petri dishes, whilst the first dilution gelatine-tube should be preserved to see if gas-bubbles develope in it.

The gelatine-plates thus prepared are frequently found to yield nothing but typhoid colonies, whilst in some cases the latter are mixed with the colonies of water-bacteria, and in some cases, again, there are only colonies of water-bacteria on the plates. In no case must it be concluded from the mere appearance of the colonies that typhoid is present, but the colonies must always be submitted to the further tests of

1. Microscopic examination.
2. Inoculation on to potatoes, and comparison of growth with that of simultaneous cultures of the typhoid bacillus on the same potatoes.
3. Inoculation into broth, and examination of the broth-culture after forty-eight hours' growth in the incubator at 37° C. for indol, which, if it is the typhoid bacillus, should be absent.
4. Inoculation into milk, which should not subsequently become coagulated on keeping for one week in the incubator.
5. Inoculation into a tube containing melted gelatine-peptone; on distributing the bacilli in this and then congealing the gelatine, no gas-bubbles should be formed on keeping at 18—20° C., whilst in the case of the *B. coli communis* bubbles will make their appearance in from twelve to forty-eight hours.

The *B. coli communis*, as already pointed out, is even less sensitive to phenol and acids than the typhoid bacillus. In the case of those unsterile waters infected with the *B. coli communis*, the above method was similarly employed for its detection.

The above outline will show that a systematic investigation of the behaviour of the typhoid bacillus in unsterilised waters is attended with considerable difficulties, and involves an enormous amount of

labour, which is, however, well worth bestowing in consideration of the very great importance attaching to the question at issue.

First Series of Experiments.

The Vitality of the Typhoid Bacillus and of the Bacillus Coli Communis *in Thames Water.*

As already indicated above, I have endeavoured to make these experiments as far as possible comparable with those previously conducted by me on the *B. anthracis* and its spores recorded in the Second Report.

The Thames water was collected by me personally from the river, close to the intake of the Grand Junction Waterworks, near Hampton, on May 4, 1893; this spot was selected as being in that region of the river from which the supply for London is abstracted.

This water was submitted to chemical analysis with the following results:—

Results of Analysis expressed in Parts per 100,000.

Total solid matters	26·80	The sample was turbid and free from poisonous metals.
Organic carbon (by combustion)	0·247	
,, nitrogen (by combustion)	0·038	
Organic nitrogen (by Kjeldahl method)	0·041	
Ammonia (free)	0·013	
,, (albuminoid)	0·016	
Oxygen consumed by organic matter	0·102	
Nitrogen as nitrates and nitrites	0·124	
Total combined nitrogen	0·173	
Chlorine	1·65	
Temporary hardness	13·8	
Permanent ,,	4·4	
Total ,,	18·2	

The analysis shows that the water is chemically a typical sample of Thames water as found in this part of the river at this season of the year.

In this series of experiments I proposed introducing the typhoid and coli bacilli respectively into (*a*) *Thames water in its natural and unsterile condition;* (*b*) *Thames water sterilised by filtration through porous porcelain;** (*c*) *Thames water sterilised by steam,*† and to com-

* The Chamberland filter (see 2nd Report, 'Roy. Soc. Proc.,' vol. liii, p. 183) was sterilised in the steamer on two successive days, 1½ litres of the Thames water being then passed through it immediately before infection.

† 1500 c.c. of the Thames water were placed in the steamer for two hours on each of three successive days.

pare the respective behaviour of the two organisms in these three different states of the Thames water.

The infection of the several waters was made on 11.5.1893, as follows :—

I. *Typhoid.*—Forty needle-loops were removed from the surface of an agar-culture of the typhoid bacillus which had been grown at 18—20° C. for fourteen days, great care being taken to carry as little as possible of the culture-material along with the growth. This growth was introduced into 50 c.c. of steam-sterilised Thames water and violently shaken for fifteen minutes in a sterile bottle to thoroughly disintegrate the masses. This water-dilution was then employed for the infection of the larger quantities of water as indicated below.

II. *B. coli.*—The water-dilution of the *B. coli communis* was made in exactly the same way as that of the typhoid bacillus described above, excepting that only twenty-five needle-loops of the growth were taken, as owing to its greater thickness it was detachable in larger masses from the surface of the agar. The culture employed was of just the same age (fourteen days), and had been grown at the same temperature (18—20° C.) as the typhoid bacillus.

With the water-dilutions thus prepared the following infections were made :—

Typhoid bacillus.	*B. coli communis.*
Unfiltered Thames Water.	
2000 c.c. received 8 c.c. of the water dilution.	1000 c.c. received 3 c.c. of the water dilution.
Porcelain-filtered Thames Water.	
750 c.c. received 3 c.c. of the water dilution.	750 c.c. received 2 c.c. of the water dilution.
Steamed Thames Water.	
750 c.c. received 3 c.c. of the water dilution.	750 c.c. received 2 c.c. of the water dilution.

The waters thus infected were each subdivided amongst a number of smaller sterilised flasks plugged with sterile cotton-wool, and these were respectively placed in the incubator or refrigerator, according as they were to be exposed to a winter or a summer temperature. Thus :

Infected.

Typhoid.	*B. Coli.*
Unfiltered Thames Water.	
3 flasks incubator.	3 flasks incubator.
3 „ refrigerator.	3 „ refrigerator.

Porcelain-filtered Thames Water.

3 flasks incubator.	3 flasks incubator.
3 ,, refrigerator.	3 ,, refrigerator.

Steamed Thames Water.

3 flasks incubator.	3 flasks incubator.
3 ,, refrigerator.	3 ,, refrigerator.

UNINFECTED CONTROL WATERS.

2 flasks incubator.
2 ,, refrigerator.

N.B.—The convention is adopted throughout the text of the Report of designating the flasks 1 *I*, 2 *I*, 3 *I*, and 1 *R*, 2 *R*, 3 *R*, according as they have been kept in the incubator or refrigerator respectively, and in this manner the individual flasks are readily identified.

In order to ascertain whether the infection of the water had communicated any large amount of organic matter to the water, some of the infected waters were submitted to a partial chemical analysis. This is a matter which has, unfortunately, been almost entirely neglected in previous observations on the vitality of pathogenic bacteria in potable waters, and it is obvious that if such investigations are to have anything but a negative value, the waters, after infection, must not differ materially in their chemical composition from that which they possess in their natural state.

These chemical analyses yielded the following results:—

Results of Analysis expressed in Parts per 100,000.

	Unfiltered Thames water* uninfected.	Unfiltered Thames water infected with typhoid.	Unfiltered Thames water infected with *B. coli*.
Ammonia (free)	0·013	0·013	0·015
,, (albuminoid)	0·016	0·020	0·028
Oxygen consumed	0·102	0·101	—
Chlorine	1·65	1·70	1·70

Thus the infection, especially in the case of the typhoid bacillus, had caused but very little increase in these ingredients.

The examination of the several flasks was conducted on the following principles:—

1. The unfiltered infected Thames water was examined by plate-cultivation from time to time in order to ascertain the changes in the

* The full analysis of this water is given on p. 409.

total number of micro-organisms present, but without any hope of counting or even identifying the typhoid or coli colonies, as this is, for the reasons already given (see pp. 406, 407) in general, quite out of the question.

On the other hand, the presence or absence of living typhoid and coli bacilli was periodically determined by cultivation with phenol-broth (see p. 407), a method which, of course, does not permit of an estimation of their number, but which, as will be seen, is often able to throw light on their relative abundance or on their relative degree of vitality.

2. The sterile (porcelain-filtered and steamed) infected Thames waters were periodically examined both by plate cultivation and by the phenol-broth method: so that in the case of these waters, in which the typhoid or coli bacilli were not mixed with other bacterial forms, not only could their presence or absence in the living state be determined, but their actual numbers ascertained.

3. The unfiltered uninfected Thames water was periodically examined by plate cultivation in order to follow the increase or decrease in the numbers of the water bacteria, whilst examinations by the phenol-broth method were also made in order to ascertain whether there were any forms amongst the water bacteria which might be confounded with the typhoid or coli bacilli, and thus to check the diagnoses made in the case of the unsterilised infected waters.

1. *Bacteriological Examination of the Uninfected Unsterilised Thames Water.* (First Series.)

It will be most convenient to consider first the behaviour of the control waters which were placed under the same conditions, in the incubator and refrigerator, as the infected ones.

The results of the gelatine plate cultivations of these control waters are summarised in the following table :—

Uninfected Unsterilised Thames Water (First Series). (Date of Collection of Sample at Hampton, 4.5.1892.)

Dates on which plate cultivations were made.	Particular flask employed.		Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies obtained from 1 c.c. of water.	
	Incubator.	Refrigerator.			Incubator flask.	Refrigerator flask.
11.5.1893	Before subdivision.		4	c.c. $\frac{1}{6}$, $\frac{1}{12}$, $\frac{1}{50}$, and $\frac{1}{100}$	290	
16.5.1893	1 I		3	$\frac{1}{50}$ and $\frac{1}{100}$	45,000	
"		1 R	3	$\frac{1}{60}$ and $\frac{1}{120}$		563,000
22.5.1893	1 I		3	$\frac{1}{60}$ and $\frac{1}{120}$	30,000	
"		I R	3	$\frac{1}{50}$ and $\frac{1}{100}$		166,000
29.5.1893	1 I		2	$\frac{1}{6}$, $\frac{1}{12}$, $\frac{1}{50}$, and $\frac{1}{100}$	28,000	
"		1 R	2	$\frac{1}{6}$, $\frac{1}{12}$, and $\frac{1}{50}$		37,000
20.6.1893	1 I		2	$\frac{1}{6}$, $\frac{1}{12}$, $\frac{1}{60}$, and $\frac{1}{120}$	44,000	
"		1 R	2	$\frac{1}{6}$, $\frac{1}{12}$, $\frac{1}{60}$, and $\frac{1}{120}$		9,000

The sample of Thames water which between the date of collection (4.5.1893) and the date of first examination (11.5.1893) had remained in bottles almost completely filled up to the stopper, and at a temperature of about 10—12° C. exhibited in the first instance an unusually small number of bacteria (only 290 in 1 c.c.). There can be little doubt that the original number present must have been greater than this, and have become diminished during this period of residence in the stoppered bottles, for on introduction into the flasks plugged with cotton-wool they underwent enormous multiplication. In the flask kept at 19° C. the multiplication was doubtless most rapid, but had already fallen again to 45,000 per c.c. on the second examination, whilst in the flask kept at 6° C. multiplication and subsequent decline were probably both less rapid, so that on the occasion of the second examination the number present was still 563,000 per c.c., which underwent continuous diminution during the remainder of the time that this flask was kept under observation.

These phenomena of initial multiplication followed by decline have been already frequently called attention to, both in the former Reports and by other observers, so that there is no necessity to dwell further upon it here beyond pointing out that it shows that the water-bacteria in this sample of water employed were in an active and flourishing state under the conditions maintained during the experiment.

Examination by Plate-Cultivation of the Unsterilised Thames Water infected with Typhoid and B. coli communis respectively.

Having in the previous pages traced the numerical changes which took place in the bacterial contents of the control uninfected unsterilised Thames water, I will now proceed to describe what occurred in the case of the same unsterilised Thames water which was infected with typhoid and coli respectively (in the manner indicated on p. 410).

The flasks containing these infected unsterilised Thames waters were kept at a winter (6—8° C.) and a summer (19° C.) temperature respectively, and were examined from time to time by gelatine plate cultivation, and the results of these periodical examinations are recorded in the following table :—

Unsterilised Thames Water infected with the Typhoid Bacillus on 11.5.1893.

Dates on which plate cultivations were made.	Particular flask employed.		Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies obtained from 1 c.c. of water.		Remarks.
	Incubator.	Refrigerator.			Incubator flask.	Refrigerator flask.	
				c.c.			
11.5.1893	Before subdivision		4	$\frac{1}{5}$, $\frac{1}{10}$, $\frac{1}{50}$, and $\frac{1}{100}$	78,000		On all the plates, almost all the colonies had the appearance of being possibly or probably typhoid, and inasmuch as the uninfected water on the same day gave only 290 colonies per 1 c.c., it is obvious that nearly all the colonies on these plates *must* have been typhoid.
16.5.1893	1 I		3	$\frac{1}{9}$, $\frac{1}{9}$, and $\frac{1}{100}$	31,000		There was a large number of liquefying colonies on these plates, but also a number of very small colonies which may very probably have been typhoid colonies. From the large number of liquefying colonies, it is obvious that the water bacteria must have undergone extensive multiplication. On the plates obtained from the very small volumes of water a large number of the colonies were easily recognisable as typhoid.
"		1 R	3	$\frac{1}{100}$ and $\frac{1}{250}$		342,000	
17.5.1893	1 I		3	$\frac{1}{120}$ and $\frac{1}{300}$	30,000		Many of the colonies were again easily recognisable as typhoid.
"		1 R	3	$\frac{1}{120}$ and $\frac{1}{300}$		485,000	

Unsterilised Thames Water infected with the Typhoid Bacillus on 11.5.1893—*continued.*

Dates on which plate cultivations were made.	Particular flask employed.		Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies obtained from 1 c.c. of water.		Remarks.
	Incubator.	Refrigerator.			Incubator flask.	Refrigerator flask.	
22.5.1893	1 I		3	c.c. $\frac{1}{50}$ and $\frac{1}{100}$	18,000		There was a large number of liquefying colonies on these plates. The recognition of typhoid colonies had become a matter of great uncertainty.
"		1 R	3	$\frac{1}{10}$, $\frac{1}{10}$, $\frac{1}{60}$, and $\frac{1}{120}$		159,000	
29.5.1893	1 I		2	$\frac{1}{10}$, $\frac{1}{10}$, $\frac{1}{50}$, and $\frac{1}{100}$	35,000		On these plates again there was a large number of liquefying colonies, also many small colonies, but recognition of typhoid involved in complete uncertainty.
"		1 R	2	$\frac{1}{12}$, $\frac{1}{12}$, $\frac{1}{60}$, and $\frac{1}{120}$		126,000	
20.6.1893	1 I		2	$\frac{1}{10}$, $\frac{1}{10}$; $\frac{1}{60}$, and $\frac{1}{120}$	22,000		Many liquefying colonies on these plates again, and no diagnosis of typhoid possible.
"		1 R	2	$\frac{1}{13}$, $\frac{1}{12}$, $\frac{1}{80}$, and $\frac{1}{160}$		5,000	

From the above table it will be seen that the unsterilised Thames water, which contained remarkably few bacteria (only 290 per c.c.) at the time, was infected with a very large number of typhoid bacilli (about 78,000 per c.c.). The total number of bacteria in the water kept at the winter temperature of 6—8° C. underwent enormous multiplication followed by decline, as in the case of the uninfected unsterilised water. In the water kept at the summer temperature of 19° C., on the other hand, the numbers found exhibited almost continuous decline, and also closely resembled those found in the uninfected unsterilised water preserved under similar conditions. In both cases, however, there must have been a great multiplication of the water-bacteria, for whilst the gelatine plates prepared from these waters during the first week after infection admitted of the ready recognition of typhoid colonies, in the subsequent examinations this was altogether impossible, so that the large number of colonies present on these later plate cultivations must have been derived from the extensive multiplication of the comparatively few water-bacteria present in this unsterilised water at the outset of the experiments.

I must, however, again emphasize what I have stated before, that whilst the recognition of typhoid colonies on such plates containing the colonies of numerous water-bacteria is often difficult and attended with much uncertainty, any estimation of the number of typhoid colonies on such plates, as has been attempted by some observers, is altogether illusory and calculated to lead to the most erroneous conclusions. For whilst the surface colonies of the typhoid bacillus are even liable to be confounded with the surface colonies of some other bacteria, in the appearance of the depth colonies (and, of course, in ordinary gelatine plates the majority of the colonies are beneath the surface) there is nothing to distinguish them from an immense number of other forms common in water. Thus, whilst in the above series of examinations I have no hesitation in saying that on the plates prepared on the 11th, 16th, and 17th May, typhoid colonies were present, I rely for the determination of their presence or absence after those dates entirely on the results of the examinations by phenol broth-culture which will be given below. Again, even in the case of those plates which obviously contained typhoid colonies, I do not consider that any estimate of their number could be justifiably made, as such an estimate could only include the surface colonies which had developed the characteristic expansions.

Thus the examination by plate-culture of these unsterilised waters does not enable us to ascertain whether the typhoid bacilli underwent any numerical increase in these waters, but from the fact that no such increase was observed in the case of the typhoid bacilli similarly introduced into steam-sterilised Thames water (see p. 451), and in which, therefore, the conditions for their multiplication were

Unsterilised Thames Water infected with the *B. coli communis* on 11.5.1893.

Date on which plate cultivations were made.	Particular flask employed.		Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies obtained from 1 c.c. of water.		Remarks.
	Incubator.	Refrigerator.			Incubator flask.	Refrigerator flask.	
11.5.1893	Before subdivision		4	c.c. $\frac{1}{50}$ and $\frac{1}{100}$	166,000		Nearly all the colonies on the plates had the typical appearance of those of *B. coli communis*.
„							
16.5.1893	1 I		3	$\frac{1}{100}$ and $\frac{1}{250}$	187,000		All the plates exhibited a large number of liquefying colonies, showing that the few water bacteria originally present must have undergone extensive multiplication. There were also a great many small colonies, doubtless to a large extent those of the *B. coli communis*.
„		1 R	3	$\frac{1}{100}$ and $\frac{1}{250}$		800,000	
23.5.1893	1 I		3	$\frac{1}{120}$ and $\frac{1}{300}$	26,000		Again a very large number of liquefying colonies present.
„		1 R	3	$\frac{1}{120}$ and $\frac{1}{300}$		321,000	
30.5.1893	1 I		2	$\frac{1}{100}$ and $\frac{1}{250}$	40,000		There were no surface colonies on these plates resembling those of the *B. coli communis*.
„		1 R	3	$\frac{1}{120}$ and $\frac{1}{300}$		7,000	
14.6.1893	2 I		2	$\frac{1}{120}$ and $\frac{1}{300}$	39,000		Flask 1 I was not used, because a little of the cotton-wool stopper got into the water on the occasion of the last examination. All the plates exhibited a large number of colonies, causing liquefaction of the gelatine, and hence necessitated early counting.
„		1 R	2	$\frac{1}{50}$ and $\frac{1}{100}$		7,500	
21.6.1893	2 I		2	$\frac{1}{120}$ and $\frac{1}{300}$	74,000		All the plates again exhibited a large number of colonies, causing liquefaction of the gelatine, and hence necessitated early counting.
„		1 R	2	$\frac{1}{100}$ and $\frac{1}{250}$		10,000	

far more favourable, there can be no reasonable doubt that they did not undergo any increase but only decline; this supposition is, moreover, corroborated by the results of the examinations by phenol broth-culture, to which I shall presently refer.

I will now turn to the similar examinations made by gelatine plate-culture of the same unsterilised Thames water infected with the *B. coli communis*, the results of which are recorded in the table on p. 418.

The results recorded in the above table for the *B. coli communis* are almost precisely parallel to those recorded in the previous table for the typhoid bacillus. There is again, in the case of the water kept at the winter temperature of 6—8° C., the enormous multiplication in the total number of bacteria present, followed by rapid and almost continuous subsequent decline. In the case of the water kept at the summer temperature of 19° C., a slight increase was observed on the occasion of the second examination (but, as pointed out in the case of the typhoid table, a great increase followed by rapid decline may have taken place in the interval between the first and second examinations), after which there was a great decline followed by some recrudescence at the end. In the case of the waters kept both at the winter and the summer temperatures respectively, however, it is obvious that extensive multiplication of the water-bacteria must have taken place, owing to the very large increase in the number of colonies causing liquefaction of the gelatine which was observed.

For the same reasons as stated in the case of the typhoid bacillus (see p. 417), it is impossible to form any estimate of the numbers in which the coli bacilli were present after the day (11.5.1893) of their introduction, nor as to the length of time over which they persisted in the living state in these waters. From the corresponding experiments, however, made with the steam-sterilised Thames water, it is quite possible that the *B. coli communis*, unlike the typhoid bacillus, may have undergone some multiplication in the water. It is to the examinations by the method of phenol broth-culture that we must again have recourse in order to ascertain how long the coli bacilli remained alive in these unsterilised waters.

There is a point which is brought out very strikingly in these tables, and to which I would draw attention at this stage, and that is that the total number of bacteria present in these unsterilised waters at the end of the period over which these experiments extended, was, both in the case of the uninfected waters (see table, p. 413) as well as in that of the typhoid (see table, p. 415), and in that of the coli (see table, p. 418), greater in the water maintained at the summer than at the winter temperature respectively. The probable explanation of this phenomenon would appear to be that at the lower temperature (6—8° C.) many of the bacteria present may be unable to form spores,

Vitality of the Typhoid Bacillus in Various Waters.

Investigators and Date of Experiments.	Source of Organism.	Temperature at which water was maintained.	Foul Water.	Ordinary Potable Water Unsterilised.	Ordinary Potable Water Sterilised.	Distilled Water.	Mineral Waters.	Sea-water or Concentrated Salt Solution.	Remarks.
Braem[1] (1889) ...	—	—	—	—	—	183 days.	—	—	*Sterilised distilled water.
Freytag[2] (1890) ...	—	—	—	—	—	—	—	5 months	Concentrated salt solution.
Giaxa[3] (1889) ...	Two days old agar-agar culture grown at 36° C.; 1 needle-point taken.	—	—	—	—	—	—	Still present on the 9th day.†	†Unsterilised sea-water.
	Two drops broth-culture 8 days old and kept at 36° C.	—	—	—	—	—	—	Still present in large numbers on the 25th day.‡	‡Sterilised sea-water.
Heraeus[4] (1886) ...	Small quantity taken from a 'streaked culture.'	37° C.	Multiplied from 2 millions to 160 millions in 2 days.*						*Unfiltered River Spree water sterilised.
		12° C.	Multiplied from 12,000 to 87,000 in 2 days.						

Hochstetter[5] (1887).	Potato-cultures grown for 4 and 7 days at temperature of room and at 36° C. Portions of the growth were mixed with sterilised distilled water and inoculated into the various waters.*	12°–15° C.	—	—	Longest duration of vitality observed, 7 days.†	Longest duration of vitality observed, 5 days.§	Longest duration of vitality observed, 5 days.¶	—	*Hochstetter states that he could detect no difference in the behaviour of the cultures grown at different temperatures. †Sterilised Berlin tap-water. §Sterilised distilled water. ¶Seltzer-water.
Hueppe[6] (1887) ...	— Taken from potato-cultures 5 days old.	10°–20° C. 15°–20° C. 10° C.	— Over 30 days; none found, however, on the 60th day.† Rapid diminution in 2 out of 5 experiments none were found on the 10th day.†	—	20 to 30 days.*	—	—	—	*Sterilised Wiesbaden tap water. †Unsterilised polluted well-water. †Ditto.
Karlinski[7] (1889)...	—	8° C.	—	6 days.*	—	—	—	—	*Unsterilised Innsbruck drinking water.
Kraus[8] (1887) ...	— —	10·5° C. 10·5° C.	— —	5 to 7 days, no longer demonstrable on 7th day.* No longer demonstrable on 7th day.†	—	—	—	—	*Unsterilised well-water. †Unsterilised Munich Mangfall water. Considered a very pure water.
Maschek[9] (1887) ...	—	18°–22° C.	—	—	10 to 80 days.*	—	—	—	Leitmeritz town water sterilised.
Mattei and Stagnitta[10] (1889)	—	8°–12° C.	—	—	4 days.				

Vitality of the Typhoid Bacillus in Various Waters—*continued.*

Investigators and Date of Experiments.	Source of Organism.	Temperature at which water was maintained.	Foul Water.	Ordinary Potable Water Unsterilised.	Ordinary Potable Water Sterilised.	Distilled Water.	Mineral Waters.	Sea-water or Concentrated Salt Solution.	Remarks.
Meade Bolton[11] (1886).	Small quantities taken from either sloped agar-agar or gelatine-cultures and mixed with sterilised ordinary salt solution from which a few drops were taken and mixed with 10 c.c. of the water under investigation.	20° C.	Over 40 days.†	—	Over 7 days.§	From 2–3 and 10–14 days. None were found between 30 and 40 days.*	—	—	*Sterilised distilled water. †Highly polluted well-water sterilised. §Ordinary Göttingen water supply, and containing very little organic matter. Sterilised.
		35° C.	From 10–14 days. None found after 20–24 or 30–40 days.†	—	—	from 2–3 days. None found after 6-7 or 10-14 or 20–24 days.*			
Pfeiffer[12] (1886) ...	—	—	—	—	Upwards of 4 months.*	—	—	—	*Sterilised well-water.
Straus and Dubarry[13] (1889).	One needle-point of a potato-culture of the bacillus introduced into 10 c.c. of the water under examination.	20° C.	—	—	32 days.* 43 days.†	30-35 days.§	—	—	*Sterilised Ourcq water. †Sterilised Vanne water. The latter has less organic matter than the Ourcq water.
		25° C. 35° C.	— —	— —	81 days * 37 days.*	69 days.§ 27 days.§			§Sterilised distilled water.
Uffelmann[14] (1888)	—	Ordinary temperature of a room.	—	2 weeks.*	—	—	—	—	*Well-water in Rostock, unsterilised.

Wolffhügel and Riedel[15] (1886).	One needle-point from a gelatine culture introduced into 50 c.c. of the water.	18°–22° C.	—	—	Over 32 days.*	—	—	—	*Sterilised Berlin tap water. †Sterilised distilled water. §Sterilised highly polluted River Panke water.
	One needle-loop from a broth-culture.	15°–20° C.	—	—	—	Over 15 days.†			
	One needle-point from a gelatine-culture introduced into 10 c.c. of the water.	35° C.	Over 10 days.§						
Slater[16] (1893) ...	Culture on agar, 37° C., 24 and also 48 hours old, inoculated into sterile distilled or sterile soda-water, 1·5 to 2 c.c. of which were employed for each inoculation.	Ordinary temperature.	—	—	—	Alive 50 days after inoculation.†	11 days, not found on the 13th day.* Dead on 5th day.‡ 8 days, dead on the 9th day.§	—	†Sterile distilled water. *Simple aërated non-sterile. ‡Sterile soda-water. §Sterile soda-water, non-aërated.

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2 "Ueber die Einwirkung concentrirter Kochsalzlösungen auf das Leben von Bakterien," 'Archiv für Hygiene,' vol. 11, 1890, p. 60.
3 "Ueber das Verhalten einiger pathogener Mikroorganismen im Meerwasser," 'Zeitschrift für Hygiene,' vol. 6, 1889, p. 162.
4 "Ueber das Verhalten der Bacterien im Brunnenwasser," 'Zeitschrift für Hygiene,' vol. 1, 1886, p. 193.
5 "Ueber Mikroorganismen im künstlichen Selterwasser," 'Arbeiten aus dem Kaiserlichen Gesundheitsamte,' vol. 2, 1887, p. 1.
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8 "Ueber das Verhalten pathogener Bakterien im Trinkwasser," 'Archiv für Hygiene,' vol 6, 1887, p. 234.
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10 "Sur la manière d'être des microbes pathogènes dans l'eau courante," 'Annali dell Istituto d'Igiene speriment. di Roma,' 1889.
11 "Ueber das Verhalten verschiedener Bakterienarten im Trinkwasser," 'Zeitschrift für Hygiene,' vol. 1, 1886, p. 76.
12 "Die Beziehung der Bodencapillarität zum Transport von Bacterien," 'Zeitschrift für Hygiene,' vol. 1, 1886, p. 398.
13 "Recherches sur la durée de la vie des microbes pathogènes dans l'eau," 'Archives de Médecine expérimentale et d'Anatomie pathologique,' vol. 1, 1889, p. 5.
14 "Trinkwasser und Infectionskrankheiten," 'Wiener medicinische Presse,' 1888, No. 37 ('Centralblatt für Bakteriologie,' vol. 5, 1889, p. 89).
15 "Die Vermehrung der Bacterien im Wasser," 'Arbeiten aus dem Kaiserlichen Gesundheitsamte,' vol. 1, 1886, p. 455.
16 "Investigation of Artificial Mineral Waters," 'Journal of Pathology and Bacteriology,' vol. 1, 1893, p. 468.

and thus perish by the long residence in the water, whilst at the higher temperature (19° C.), although the fully developed bacteria are more rapidly destroyed, a larger proportion of them give rise to spores and thus lead to a larger permanent bacterial population in the water.

Examination of the Unsterilised Thames Waters, Infected and Uninfected by Phenol Broth-culture.

I must now call attention to the results of the phenol broth-cultivations made both with the unsterilised Thames water, as well as with that infected with the typhoid bacillus and the *B. coli communis* respectively.

The following experiment will show how under favourable conditions, the phenol broth test serves to distinguish a water containing typhoid bacilli from another in which they are absent; thus

Phenol Broth Experiments (12.5.1893).

(1) 1 c.c. uninfected unsterilised Thames water added to 10 c.c. broth + 5 drops phenol solution.
(2) 1 c.c. uninfected unsterilised Thames water added to 10 c.c. broth + 3 drops phenol solution.
(3) 0·5 c.c. uninfected unsterilised Thames water added to 10 c.c. broth + 5 drops phenol solution.
(4) 0·5 c.c. uninfected unsterilised Thames water added to 10 c.c. broth + 3 drops phenol solution.
(5) 1 c.c. unsterilised Thames water infected with typhoid added to 10 c.c. broth + 5 drops phenol solution.
(6) 1 c.c. unsterilised Thames water infected with typhoid added to 10 c.c. broth + 3 drops phenol solution.
(7) 0·5 c.c. unsterilised Thames water infected with typhoid added to 10 c.c. broth + 5 drops phenol solution.
(8) 0·5 c.c. unsterilised Thames water infected with typhoid added to 10 c.c. broth + 3 drops phenol solution.

These eight tubes, all in duplicate, were placed in an incubator at 38° C., and on the following day, whilst all the uninfected tubes, 1, 2, 3, and 4, were clear, all the infected tubes, 5, 6, 7, and 8, were turbid, thus showing that whilst the addition of the phenol solution had prevented the proliferation of the ordinary water-bacteria in the uninfected Thames water, the extensive multiplication of the typhoid bacilli in the infected Thames water had taken place in spite of the presence of the same proportions of phenol. In the same way, on the following day, similar quantities of the unsterilised Thames water infected with the *B. coli communis* were introduced into broth-

tubes, to which the same proportions, as above, of phenol solution were added, and all these tubes similarly became turbid on being kept at 38° C. for twenty-four hours.

Thus at the outset of this series of experiments, the uninfected Thames water was sharply distinguishable by means of the phenol broth test from the same Thames water after infection with either the typhoid bacillus or the *B. coli communis*.

The uninfected and infected unsterilised Thames waters were again compared by the method of phenol broth cultivation on 29.5.1893.

Number of broth tube.	Water used.	Quantity of water taken. c.c.	Quantity of phenol solution added to 10 c.c. broth.	Remarks.
	Unsterilised Uninfected Thames.			
(1)	Flask 1 I ..	0·5	5 drops	No turbidity even on 6.6.1893.
(2)	" ..	1·0	"	
(3)	Flask 1 R ..	0·5	"	
(4)	" ..	1·0	"	
	Unsterilised Typoid-infected Thames.			
(5)	Flask 1 I ..	0·5	"	Did not become turbid.
(6)	" ..	1·0	"	Turbid in 48 hours.
(7)	Flask 1 R ..	0·5	"	Turbid in 24 hours.
(8)	" ..	1·0	"	" "

The results recorded in the above table indicated that on 29.5.1893, whilst there were still no bacteria in the uninfected unsterilised Thames water to interfere with the phenol broth test, this test pointed to the presence of living typhoid bacilli in the typhoid-infected Thames waters, which had been kept both at 6° C. and at 19° C. (flasks 1 R and 1 I). The results of the test, moreover, indicate that these typhoid bacilli were now less numerous or in a less active condition in the flask 1 I (19° C.) than in the flask 1 R (6° C.), because both phenol broth tubes prepared from 1 R became turbid already in twenty-four hours, whilst of the two similar tubes prepared from flask 1 I, only the one in which 1 c.c. of water was employed for cultivation

became turbid, and then only after forty-eight hours, whilst the tube in which only 0·5 c.c. of water was employed did not become turbid at all.

It must not, however, be supposed that the diagnosis of typhoid bacilli in these waters was allowed to rest on such slender evidence as the mere clouding of these phenol broth-cultures, but the latter were submitted to gelatine plate cultivation to see if the characteristic typhoid colonies made their appearance, and these colonies were further confirmed by inoculation (*a*) on to potatoes for exhibition of the characteristic growth, (*b*) into gelatine tubes to see if bubbles of gas would make their appearance, (*c*) into broth for the indol test, and generally also (*d*) into milk to see whether coagulation of the casein would take place. Thus in the case of the above phenol broth-cultures commenced on 29.5.1893, the final confirmation of typhoid was not obtained until 12.6.1893, or a fortnight later.

The phenol broth test was again applied to the waters on 5.6.1893, with the following results (p. 427).

The plate cultivations made from the phenol broth tubes, referred to in the table (p. 427), yielded the following results :—

Broth tube.

(21.) *Typhoid-infected Unsterilised Thames, Flask* 1 I. (*Typhoid Present.*)

The presence of typhoid was confirmed by the typical appearance of colonies, growth on potatoes, negative indol test, and negative gelatine bubble test.

(23.) *Typhoid-infected Unsterilised Thames, Flask* 1 R. (*Typhoid Present.*)

The presence of typhoid was confirmed by the typical colonies, growth on potatoes, negative indol, and negative gelatine bubble tests.

(37.) and (45.) *Coli-infected Unsterilised Thames, Flask* 2 I. (*B. coli Present.*)

The presence of the *B. coli* was confirmed by typical colonies, growth on potatoes, positive indol, and positive gelatine bubble tests.

(39.) and (47.) *Coli-infected Unsterilised Thames, Flask* 1 R. (*B. coli Present.*)

The presence of the *B. coli* was confirmed by typical colonies, growth on potatoes, positive indol, and positive gelatine bubble tests.

Examination by Phenol Broth-culture on 5.6.1893.

Number of broth tube.	Water used for cultivation with phenol broth.	Quantity of water taken in c.c.	Quantity of phenol solution added to 10 c.c. broth.	Remarks.
		Unsterilised Typhoid-infected Thames.		
(21)	Flask 1 I ..	0·5	3 drops	Turbid in 48 hours. Plates poured 9.6.1893.
(22)	,, ..	1·0	,,	Turbid in 48 hours.
(23)	Flask 1 R ..	0·5	,,	Turbid in 24 hours. Plates poured 6.6.1893.
(24)	,, ..	1·0	,,	Turbid in 24 hours.
		Unsterilised Coli-infected Thames.		
(37)	Flask 2 I ..	0·5	3 drops	Turbid in 24 hours. Plates poured 6.6.1893.
(38)	,, ..	1·0	,,	Turbid in 24 hours.
(39)	Flask 1 R ..	0·5	,,	Turbid in 24 hours. Plates poured 6.6.1893.
(40)	,, ..	1·0	,,	Turbid in 24 hours.
		Unsterilised Uninfected Thames.		
(41)	Flask 1 I ..	0·5	3 drops	Turbid in 48 hours. Plates poured 9.6.1893.
(42)	,, ..	1·0	,,	Turbid in 48 hours.
(43)	Flask 1 R ..	0·5	,,	Turbid in 48 hours. Plates poured 9.6.1893.
(44)	,, ..	1·0	,,	Turbid in 48 hours.
		Unsterilised Coli-infected Thames.		
(45)	Flask 2 I ..	0·5	5 drops	Turbid in 24 hours. Plates poured 6.6.1893.
(46)	,, ..	1·0	,,	Turbid in 24 hours.
(47)	Flask 1 R ..	0·5	,,	Turbid in 24 hours. Plates poured 6.6.1893.
(48)	,, ..	1·0	,,	Turbid in 24 hours.

Broth tube.

(41.) *Uninfected Unsterilised Thames, Flask* 1 I.

The only colonies on the plate which bore any resemblance to typhoid, yielded pink growths on potatoes, and developed green fluorescence on being grown in gelatine tubes; they were thus in reality wholly unlike and different from typhoid or coli.

Broth tube.

(43.) *Uninfected Unsterilised Thames, Flask* 1 R.

Colonies bearing some resemblance to typhoid yielded growths on potatoes, which were also not unlike typhoïd, but on inoculation into gelatine tubes a green fluorescence was obtained, conclusively proving that it was another organism and not typhoid or coli.

From these examinations then it was apparent that on this day, 5.6.1893,—

1. *The unfiltered Thames water infected with* typhoid *on* 11.5.1893, *or twenty-five days previously, still contained living typhoid bacilli, both in that portion of the water which had been preserved at* 19° *C. as well as in that kept at* 6° *C., the number and vital activity of the typhoid bacilli being apparently greater in the latter than in the former.*

2. *The unfiltered Thames water infected with the* B. coli communis *on the same date also still contained these bacilli in a living state, both in that portion of the water kept at* 19° *C. as well as in that maintained at* 6° *C.*

3. *The unfiltered uninfected Thames water which had been maintained under exactly similar conditions, contained no bacteria which after careful examination could be mistaken either for the typhoid bacillus or the* B. coli communis.

The unfiltered infected waters were again examined on 14.6.1893, with the following results:—

Examination by Phenol Broth-culture on 14.6.1893.

Number of broth tube.	Water used for cultivation with phenol broth.	Quantity of water taken in c.c.	Quantity of phenol solution added to 10 c.c. broth.	Remarks.
		Unsterilised Typhoid-infected Thames.		
(85)	Flask 1 I ..	1·0	3 drops	Turbid in 48 hours. Plates poured.
(86)	Flask 1 R..	1·0	,,	,, ,,
		Unsterilised Coli-infected Thames.		
(87)	Flask 1 I ..	1·0	3 drops	Turbid in 24 hours. Plates poured.
(88)	Flask 1 R..	1·0	,,	,, ,,

The plate cultivations made from the above phenol broth tubes gave the following results:—

Broth tube.

(85.) *Unsterilised Typhoid-infected Thames, Flask* 1 I. (*Typhoid Absent.*)

The plate exhibited many liquefying colonies as well as a large number of very small colonies; the latter were placed on potatoes, and yielded light brown growths; on inoculation into gelatine tubes liquefaction took place, therefore certainly not typhoid.

(86.) *Unsterilised Typhoid-infected Thames, Flask* 1 R. (*Typhoid Absent.*)

The small colonies on the plate which alone exhibited *any* resemblance to typhoid were examined as in No. 85 above, and yielded exactly similar results, therefore certainly not typhoid.

(87.) *Unsterilised Coli-infected Thames, Flask* 1 *Incubator.* (*B. coli Present.*)

Almost pure cultivation, with numerous typical extension colonies like *B. coli;* these yielded characteristic growth on potatoes, gave the indol reaction, and the gas-bubbles in gelatine tube. *B. coli*, therefore, present.

(88.) *Unsterilised Coli-infected Thames, Flask* 1 *Refrigerator.* (*B. coli Present.*)

Exactly similar results with this as with No. 87 above. *B. coli*, therefore, present.

From these examinations it appears that on 14.6.1893,—

1. *The* typhoid *bacillus was no longer demonstrable in the unsterilised Thames water, which had been infected with it thirty-four days previously. It had disappeared both in that portion of the water which had been preserved at a winter, as well as in that kept at a summer, temperature.*

2. *The* B. coli communis, *on the other hand, was easily demonstrable in similar water which had been preserved under precisely the same conditions.*

These infected unsterilised Thames waters were again submitted to examination on 21.6.1893, with the following results:—

Examination by Phenol Broth-culture on 21.6.1893.

Number of broth tube.	Water used for cultivation with phenol broth.	Quantity of water taken in c.c.	Quantity of phenol solution added to 10 c.c. broth.	Remarks.
		Typhoid-infected Unsterilised Thames.		
(86A)	Flask 1 I ..	1·0	3 drops	Turbid in 48 hours. Plates poured 23.6.1893.
(87A)	Flask 1 R ..	1·0	„	Not turbid on 4.7.1893.
		Coli-infected Unsterilised Thames.		
(94)	Flask 2 I ..	1·0	3 drops	Turbid in 24 hours. Plates poured 22.6.1893.
(95)	Flask 1 R ..	1·0	„	„ „ „ „

The plate cultivations made from the above phenol broth tubes gave the following results:—

Broth tube.

(86A.) *Typhoid-infected Unsterilised Thames, Flask 1 Incubator. (Typhoid Absent.)*

The colonies were inoculated on to potatoes, on which a light brown growth extending over the whole potato was obtained. On inoculation into gelatine tubes liquefaction followed, therefore certainly not typhoid. No bubbles of gas appeared in the gelatine.

(94.) *Coli-infected Unsterilised Thames, Flask 2 Incubator. (B. coli Present.)*

The plate had the appearance of being a pure cultivation of *B. coli*, with the characteristic colonies, yielding characteristic growth on potatoes, also indol reaction, and gas bubbles on inoculation into melted gelatine tubes. *B. coli*, therefore, present.

(95.) *Coli-infected Unsterilised Thames, Flask 1 Refrigerator. (B. coli Present.)*

Exactly similar results were obtained with this as with No. 94 above. *B. coli*, therefore, present.

Thus, on this day (21.6.1893) *as on the occasion of the previous examination* (14.6.1893), *the typhoid bacilli were no longer demonstrable in the unsterilised Thames water into which they had been introduced on* 11.5.1893. *The* B. coli communis, *on the other hand, was again easily discoverable under the same circumstances,* i.e., *forty days after its introduction into the unsterilised Thames water.*

The typhoid-infected unsterilised Thames water was again similarly examined on 26.6.1893, with the following results :—

Examination by Phenol Broth-culture on 26.6.1893.

Number of broth tube.	Water used for cultivation with phenol broth.	Quantity of water taken in c.c.	Quantity of phenol solution added to 10 c.c. broth.	Remarks.
		Typhoid-infected Unsterilised Thames.		
(96)	Flask 1 I ..	1·0	3 drops	Turbid in 48 hours. Plates poured.
(97)	Flask 1 R ..	1·0	"	" " " "

The plate cultivations made from the above phenol broth tubes yielded the following results :—

Broth tube.

(96.) *Typhoid-infected Unsterilised Thames, Flask* 1 *Incubator.* (*Typhoid Absent.*)

The plates contained some small colonies presenting some resemblance to typhoid ; on being transferred to potatoes they yielded light brown growths unlike typhoid, and on being inoculated into gelatine green fluorescence without liquefaction was obtained. These colonies were, therefore, not those of typhoid.

(97.) *Typhoid-infected Unsterilised Thames, Flask* 1 *Refrigerator.* (*Typhoid Absent.*)

The plates exhibited two types of colony, firstly, liquefying ones which could not be typhoid, and, secondly, small depth colonies, which on transference to potatoes gave thick greyish-brown growths wrinkled in parts, and on inoculation into gelatine tubes caused subsequent liquefaction. These were, therefore, not typhoid.

This examination, made on 26.6.1893, *confirmed, therefore, the two previous examinations of* 21.6.1893 *and* 14.6.1893, *which showed that the typhoid bacillus, introduced on* 11.5.1893, *was no longer discoverable in the unsterilised Thames water.*

The final examination of these waters was made on 5.7.1893, thus—

Examination by Phenol Broth-culture on 5.7.1893.

Number of broth tube.	Water used for cultivation with phenol broth.	Quantity of water taken in c.c.	Quantity of phenol solution added to 10 c.c. broth.	Remarks.
		Unsterilised Uninfected Thames.		
(106)	Flask 1 I ..	1·0	3 drops	Turbid in 48 hours, Plates poured 7.7.1893.
(107)	„ 1 R.	1·0	„	Ditto.
(119)	Flask 1 I ..	1·0	5 drops	Not turbid in 6 days.
(120)	„ 1 R..	1·0	„	Ditto.
		Typhoid infected Unsterilised Thames.		
(108)	Flask 1 I ..	1·0	3 drops	Turbid in 72 hours. Plates poured 8.7.1893.
(109)	„ 1 R..	1·0	„	Turbid in 48 hours. Plates poured 7.7.1893.
(121)	Flask 1 I ..	1·0	5 drops	Not turbid in 6 days.
(122)	„ 1 R..	1·0	„	Ditto.

Thus, whilst the broth tubes containing only 3 drops of phenol still became turbid when inoculated with both uninfected and infected waters, the broth tubes to which 5 drops of the phenol solution were added remained clear after inoculation with both waters.

The plate cultivations made from the above broth-tubes, which became turbid, yielded the following results :—

Broth tube.

(106.) *Unsterilised Uninfected Thames, Flask* 1 *Incubator.*

The plates exhibited a large number of highly fluorescent expansion colonies without liquefaction; some of the least fluorescent of these colonies, and bearing therefore a faint resemblance to typhoid, were transferred to potatoes, on which they gave rise to light brown, sharply demarcated growths, quite unlike typhoid. No gas bubbles were produced on inoculation into melted gelatine tubes.

Broth tube.

(107.) *Unsterilised Uninfected Thames Flask 1 Refrigerator.*

The plates exhibited an apparently pure cultivation of a liquefying organism, the colonies having a strong resemblance to those of *B. liquidus* (see 2nd Report, p. 186). Some of the colonies which had not yet caused liquefaction were transferred to potatoes, on which they gave rise to thick, whitish, waxy growths, quite unlike those of typhoid.

(108.) *Typhoid-infected Unsterilised Thames, Flask 1 Incubator.* (*Typhoid Absent.*)

The plates contained fluorescent expansion, liquefying and small dot colonies. All three types of colony were transferred to potatoes, on which the small dot and fluorescent expansion colonies gave rise to light brown sharply marked growths, and the liquefying colonies to shining slimy colourless growths; none of these were, therefore, similar to those of typhoid.

(109). *Typhoid-infected Unsterilised Thames, Flask 1 Refrigerator.* (*Typhoid Absent.*)

The plates appeared to be pure cultivations; the depth colonies were small dots, often oval in shape, and the surface colonies resembled very small "milk-drops;" even on the plate in which the colonies were few and far between there were none of the typical expansion colonies resembling typhoid. On transference to potatoes, shining slimy growths, having the appearance of drops of water, were obtained.

This final examination of these unsterilised typhoid-infected Thames waters therefore again confirmed the previous results, which may be thus summarised:—

(1.) The unsterilised uninfected Thames water, collected at Hampton on 5.5.1893, contained throughout the entire course of the series of experiments no bacteria resembling either the typhoid bacillus or the *B. coli communis*.

(2.) The unsterilised Thames water infected with typhoid on 11.5.1893, was still found to contain living typhoid bacilli on 5.6.1893, or twenty-five days after infection, whilst on 14.6.1893, thirty-four days after infection, they were no longer demonstrable.

(3.) These remarks apply equally to the waters preserved at a winter and a summer temperature respectively,

although there is some evidence that on the last day of their detection the typhoid bacilli were present, either in larger numbers or in a more active state in the water maintained at the winter than in that at the summer temperature.

(4.) The same unsterilised Thames water infected with the *B. coli communis* on the same day, and kept under precisely similar conditions of temperature, &c., was still found to contain living coli bacilli forty days after their introduction. These bacilli doubtless persisted in the living state, even for a much longer period of time than this, no later examinations being made; and on the occasion of their last detection they did not appear to have lost any of their original vitality, as they still promptly reacted with the phenol broth test.

Experiments on the Influence of the Addition of Salt to the Unsterilised Typhoid-infected Thames Water.

In the recent cholera epidemic at Hamburg, it is now almost universally recognised that the most important agent in distributing the zymotic poison was the highly polluted and unfiltered water of the River Elbe. This water, moreover, during the epidemic was found to be unusually rich in salt, in fact at times it was distinctly brackish in character. Thus a sample of Hamburg water sent to me by Mr. Ernest Hart in October, 1892, and which I submitted to analysis, had the following composition:—

Sample of Hamburg Water received from Mr. Ernest Hart.

Results of Analysis expressed in Parts per 100,000.

Total solid matters.	Organic carbon.	Organic nitrogen.	Ammonia.		Nitrogen as nitrates and nitrites.	Chlorine.	Hardness.		
			Free.	Albuminoid.			Temporary.	Permanent.	Total.
78·00	0·926	0·088	0·030	0·047	0	31·3	4·1	13·7	17·8

Oxygen consumed by organic matter, as measured by reduction of a solution of permanganate acting for three hours in the cold = 0·366.

The water was very turbid, depositing a quantity of brown suspended matter.

The high percentage of salt is due to the waste liquors which are discharged from the Stassfurth salt works and other factories into the Elbe and its tributaries. Now it has been more recently shown (Trenkmann, "Beitrag zur Biologie des Kommabacillus," 'Centralbl

f. Bakteriologie,' vol. 13 (1893), p. 313) that the addition of sodium chloride, and of other salts in certain proportions, to water containing the cholera bacilli causes a most remarkable multiplication of the latter, as may be seen from the following table, given by Trenkmann, which exhibits the effect of making such additions to a sterilised well water, which had been purposely infected with cholera bacilli.

Addition of various Salts to Sterilised Well Water containing Cholera Bacilli.

The waters were maintained at 21—24° C.	(Trenkmann.) Number of cholera bacilli in 1 needle loop. After 24 hours.	After 8 days.
(1.) 10 c.c. sterile well water	580 520	5
(2.) ,, + 1 drop* 10 per cent. sodium chloride	6,120	12,480
(3.) ,, + 2 drops ,, ,, ,,	9,240	19,560
(4.) ,, + 3 ,, ,, ,, ,,	15,000	10,440
(5.) ,, + 1 drop ,, ,, nitrite	1,740	10,920
(6.) ,, + 2 drops ,, ,, ,,	6,600	1,460
(7.) ,, + 3 ,, ,, ,, ,,	17,160	2,260
(8.) ,, + 1 drop ,, ,, nitrate	8,040	4,040
(9.) ,, + 2 drops ,, ,, ,,	6,660	14,760
(10.) ,, + 3 ,, ,, ,, ,,	20,940	16,080
(11.) ,, + 1 drop ,, disodium phosphate	3,360	—
(12.) ,, + 2 drops ,, ,, ,,	7,560	—
(13.) ,, + 3 ,, ,, ,, ,,	6,540	—
(14.) ,, + 1 drop ,, sodium carbonate	7,440	—
(15.) ,, + 2 drops ,, ,, ,,	28,680	—
(16.) ,, + 3 ,, ,, ,, ,,	31,560	—
(17.) ,, + 2 ,, ,, ,, chloride and 1 drop 10 per cent. disodium phosphate	54,720	—

* 25—27 drops = 1 c.c.

These results show that the presence of an unusually high proportion of salts may not improbably have played an important part in the distribution of cholera by means of the water of the Elbe in the recent Hamburg epidemic, and possibly also by means of the Thames water in some of the former London epidemics, as at that time the metropolitan supply was in part derived from the tidal portion of the river.

In view of these circumstances, it appeared to me to be of considerable interest to ascertain whether and in what way the behaviour of the typhoid bacillus in water is affected by additions of salt, and, with this object, the following experiments were undertaken :—

Preparation of the Saline Waters.—The saline waters employed were of three different strengths—

(*a*.)	Containing	0·1	per cent.	sodium chloride.
(*b*.)	„	1·0	„	„
(*c*.)	„	3·0	„	„

Three portions of pure sodium chloride, weighing respectively 0·5, 5·0, and 15·0 grams, were sterilised in the air-oven at 150° C. for several hours, and then placed in three sterile 500 c.c. measuring flasks. To each of these was added sufficient of the typhoid-infected unsterilised Thames water (p. 410) to dissolve the salt, and the solution was in each case further diluted to the 500 c.c. mark with the same infected unsterilised Thames water. These saline waters were then distributed in smaller flasks plugged with cotton-wool, and two of the latter of each particular strength were placed in the refrigerator at 6—8° C., and two in the incubator at 19° C. Thus there were

Unsterilised typhoid-infected Thames water +0·1 per cent. salt	2 flasks	refrigerator
	2 „	incubator
Unsterilised typhoid-infected Thames water +1·0 per cent. salt	2 flasks	refrigerator
	2 „	incubator
Unsterilised typhoid-infected Thames water +3·0 per cent. salt	2 flasks	refrigerator
	2 „	incubator

These saline waters, like the others, were prepared on 11.5.1893, and were examined by gelatine plate cultivation at frequent intervals subsequently. The results obtained must obviously be compared with those from the typhoid-infected unsterilised Thames water, to which no salt was added, and which have already been recorded in the Table on pp. 415 and 416, but which are again given, so as to facilitate comparison, in the first column of the following table :—

Typhoid-infected Unsterilised Thames Water, with and without the addition of Common Salt. Experiments commenced 11.5.1893.

Dates on which plate cultivations were made.	Number of colonies obtained from 1 c.c.							
	Water without additions.		Water + 0·1 per cent. NaCl.		Water + 1·0 per cent. NaCl.		Water + 3·0 per cent. NaCl.	
	Incubator flask.	Refrigerator flask.	Incubator flask.	Refrigerator flask.	Incubator flask.	Refrigerator flask.	Incubator flask.	Refrigerator flask.
11.5.1893	78,000 (4 days)*	78,000	(78,000)	(78,000)	(78,000)	(78,000)	(78,000)	(78,000)
17.5.1893	30,000 (3 days)	485,000 (3 days)	189,000 (3 days)	381,000 (3 days)	366,000 (3 days)	436,000 (3 days)	3,900,000 (3 days)	300 (8 days)
22.5.1893	18,000 (3 days)	159,000 (3 days)	33,000 (3 days)	262,000 (3 days)	56,000 (3 days)	1,800,000 (3 days)	3,000,000 (4 days)	194,000 (5 days)
29.5.1893	35,000 (2 days)	126,000 (2 days)	71,000 (2 days)	143,000 (2 days)	53,000 (2 days)	1,500,000 (2 days)	5,000,000 (4 days)	2,000,000 (4 days)
20.6.1893	22,000 (2 days)	5,000 (2 days)	43,000 (2 days)	9,000 (2 days)	31,000 (2 days)	110,000 (2 days)	3,600,000 (3 days)	1,700,000 (2 days)

* This refers to the number of days that the gelatine plates were incubated at 18—20° C.

From the above table it will be seen that the addition of common salt to the typhoid-infected unsterilised Thames water occasioned an enormous increase in the number of water-bacteria, the effect being most pronounced in the case of the water to which 3 per cent. addition had been made, whilst the water which had received an addition of only 0·1 per cent. of sodium chloride gave results which did not differ materially from those yielded by the water to which no salt had been added.

The addition of salt was by no means conducive to the welfare of all the different kinds of water-bacteria present, but, on the contrary, from the appearance of the plate cultivations, it was evident that some forms were favoured at the expense of others; thus, on the plates prepared from the 3 per cent. salt water, there was a conspicuous absence of liquefying colonies, these plates having, in fact, almost the appearance of pure cultivations. This entirely bears out what I found a number of years ago ("On the Multiplication of Micro-organisms," 'Proc. Roy. Soc.,' 1886), that in a water containing only a very limited number of species there is generally far more extensive multiplication than in the case of one containing many different species.

The manner in which the salt operated is most apparent from that flask of the water to which an addition of 3 per cent. had been made, and which was preserved in the refrigerator (6—8° C.), as in this case the multiplication took place most slowly. Thus, whilst the water at the outset contained 78,000 bacteria per c.c., of which nearly all were typhoid bacilli, after six days there were only 300 bacteria per c.c., so that there must have been an enormous destruction in the interval, but the bacteria left subsequently underwent enormous multiplication, although more slowly than in the case of the corresponding flask kept in the incubator (19° C.).

In all cases it will be seen that the multiplication was followed by decline, but this decline was greatly retarded in those waters which had received the large additions of salt.

We must now consider the effect of these additions of salt on the typhoid bacilli in the waters, as indicated by the results of phenol broth-culture.

These saline waters were first submitted to phenol broth-culture on 29.5.1893, with the following results :—

Examination by Phenol Broth-culture, 29.5.1893.

Number of broth tube.	Water used for cultivation with phenol broth.	Quantity of water taken in c.c.	Quantity of phenol solution added to 10 c.c. broth.	Remarks.
		Typhoid-infected Unsterilised Thames + 0·1 per cent. NaCl.		
(9)	Flask 1 I ..	0·5	5 drops	Did not become turbid even in seven days.
(10)	" ..	1·0	"	" " "
(11)	Flask 1 R ..	0·5	"	Turbid in 24 hours. Plates poured 30.5.1893.
(12)	" ..	1·0	"	" " "
		Typhoid-infected Unsterilised Thames + 1·0 per cent. NaCl.		
(13)	Flask 1 I ..	0·5	5 drops	Did not become turbid even in seven days.
(14)	" ..	1·0	"	" " "
(15)	Flask 1 R ..	0·5	"	" " "
(16)	" ..	1·0	"	" " "
		Typhoid-infected Unsterilised Thames + 3 per cent. NaCl.		
(17)	Flask 1 I ..	0·5	5 drops	Did not become turbid even in seven days.
(18)	" ..	1·0	"	" " "
(19)	Flask 1 R ..	0·5	"	" " "
(20)	" ..	1·0	"	" " "

Thus, the only saline water which gave a positive reaction with the phenol broth test was the one in which an addition of only 0·1 per cent. of salt had been made, and, even in this case, it was only the flask which had been kept at the winter temperature of 6—8° C. in the refrigerator, whilst the corresponding flask preserved at the summer temperature of 19° C. gave a negative result. Plate cultivations were made of the turbid broth-tube No. 11, and these yielded the characteristic typhoid colonies, which were further confirmed by potato growth, and negative results with the indol and gas-bubble tests. On referring to the results of phenol broth-culture (pp. 424—434) obtained with the corresponding waters to which no salt had been added, it will be seen that they present a great contrast to these, as both the incubator and refrigerator flasks of the typhoid-infected unsterilised Thames water gave a positive reaction, and were found to contain living typhoid bacilli.

From these examinations it appears then that on 29.5.1893, *or eighteen*

days after infection, the unsterilised Thames water still contained living typhoid bacilli, as did also the same water to which 0·1 *per cent. common salt had been added, and which had been preserved at* 6—8° C.; *on the other hand, in the unsterilised Thames waters to which* 1·0 *and* 3·0 *per cent. respectively of salt had been added, as well as in that which had only received* 0·1 *per cent. salt, but which had been kept at* 19° C., *the typhoid bacilli were no longer demonstrable.*

These saline waters were again examined by phenol broth-culture on 5.6.1893, with the following results:—

Examination by Phenol Broth-culture, 5.6.1893.

Number of broth tube.	Water used for cultivation with phenol broth.	Quantity of water taken in c.c.	Quantity of phenol solution added to 10 c.c. broth.	Remarks.
				Typhoid-infected Unsterilised Thames + 0·1 *per cent.* NaCl.
(25)	Flask 1 I ..	0·5	3 drops	Turbid in 48 hours (pellicle on broth). Plates poured 9.6.1893.
(26)	,, ..	1·0	,,	Turbid in 48 hours.
(31)	Flask 1 R ..	0·5	,,	Turbid in 24 hours. Plates poured 6.6.1893.
(32)	,, ..	1·0	,,	Turbid in 24 hours.
				Typhoid-infected Unsterilised Thames + 1 *per cent.* NaCl.
(27)	Flask 1 I ..	0·5	3 drops	Turbid in 48 hours. Plates poured 9.6.1893.
(28)	,, ..	1·0	,,	Turbid in 48 hours.
(33)	Flask 1 R ..	0·5	,,	Turbid in 72 hours. Plates poured 9.6.1893.
(34)	,, ..	1·0	,,	Turbid in 48 hours.
				Typhoid-infected Unsterilised Thames + 3 *per cent.* NaCl.
(29)	Flask 1 I ..	0·5	3 drops	Not turbid until 8 days. Plates poured 13.6.1893.
(30)	,, ..	1·0	,,	Turbid in 72 hours (pellicle on broth). Plates poured 9.6.1893.
(35)	Flask 1 R ..	0·5	,,	Not turbid until 8 days. Plates poured 13.6.1893.
(36)	,, ..	1·0	,,	Not turbid until 8 days.

Thus from the above the only water in which the presence of typhoid was still with certainty to be expected was the one to which 0·1 per cent. salt had been added, and which had been kept at 6—8° C. in the refrigerator, for this was the only one which gave a positive

result with the phenol broth in twenty-four hours, and although several others gave the reaction in forty-eight hours, it will be seen, by reference to p. 427, that even the uninfected unsterilised Thames water gave the turbidity in forty-eight hours when examined on the same day.

The plate cultivations made from the above turbid broth tubes gave the following results:—

Broth tube.

(31.) *Typhoid-infected Unsterilised Thames* + 1 *per cent.* NaCl, *Flask* 1 *Refrigerator.* (*Typhoid Present.*)

The colonies on the plate resembled those of typhoid, and they were confirmed by potato growth, negative indol, and gas-bubble tests.

(35.) *Typhoid-infected Unsterilised Thames* + 3 *per cent.* NaCl, *Flask* 1 *Refrigerator.* (*Typhoid Absent.*)

The colonies presenting any sort of resemblance to those of typhoid were transferred to potatoes, on which they gave a pink growth, therefore certainly not typhoid.

(29.) *Typhoid-infected Unsterilised Thames* + 3 *per cent.* NaCl, *Flask* 1 *Incubator.* (*Typhoid Absent.*)

Results similar to those obtained with No. 35 above.

(25.) *Typhoid-infected Unsterilised Thames* + 0·1 *per cent.* NaCl, *Flask* 1 *Incubator.* (*Typhoid Absent.*)

Results similar to those obtained with Nos. 35 and 29 above.

(27.) *Typhoid-infected Unsterilised Thames* + 1 *per cent.* NaCl, *Flask* 1 *Incubator.* (*Typhoid Absent.*)

The colonies presented some resemblance to those of typhoid, as did also the potato growths; on inoculating from latter, however, into gelatine tube, the gelatine underwent slow liquefaction, clearly showing that it was not really typhoid.

(33.) *Typhoid-infected Unsterilised Thames* + 1 *per cent.* NaCl, *Flask* 1 *Refrigerator.* (*Typhoid Absent.*)

The colonies which presented any sort of resemblance to those of typhoid were transferred to potatoes, on which they gave a pink growth, and, on inoculating into gelatine tubes, gas bubbles were formed, followed by liquefaction. Therefore, certainly not typhoid.

Broth tube.

(30.) *Typhoid-infected Unsterilised Thames* + 3 *per cent.* NaCl, *Flask* 1 *Incubator.* (*Typhoid Absent.*)

The colonies presented *some* resemblance to typhoid, so they were further examined by potato growth, indol, gas-bubble, and milk tests, in all of which the resemblance was maintained. Microscopically, the bacilli appeared smaller than typhoid, but the difference was not sufficient to place the matter beyond doubt. They were finally proved not to be typhoid by keeping the gelatine tube cultures for some time, when both the surface and depth growths were found to be distinctly yellow in colour. I have repeatedly encountered this same organism, which might be mistaken for typhoid in its earlier appearance in plate cultures, but it must clearly be understood that the resemblance is only a superficial one, and it was only submitted to so many tests because in these examinations anything bearing the slightest resemblance to typhoid was remanded for further enquiries.

These saline waters were again examined by phenol broth-culture on 13.6.1893, with the following results:—

Examination by Phenol Broth-culture on 13.6.1893.

Number of broth tube.	Water used for cultivation with phenol broth.	Quantity of water taken in c.c.	Quantity of phenol solution added to 10 c.c. broth.	Remarks.
		Typhoid-infected unsterilised Thames + 0·1 *per cent.* NaCl.		
(73)	Flask 1 I ..	1·0	3 drops	Turbid in 24 hours. Plates poured 14.6.1893.
(74)	,, ..	0·5	,,	Turbid in 48 hours.
(79)	Flask 1 R ..	1·0	,,	Turbid in 24 hours. Plates poured 14.6.1893.
(80)	,, ..	0·5	,,	Turbid in 48 hours.
		Typhoid-infected unsterilised Thames + 1·0 *per cent.* NaCl.		
(75)	Flask 1 I ..	1·0	3 drops	Turbid in 24 hours. Plates poured 14.6.1893.
(76)	,, ..	0·5	,,	Turbid in 48 hours.
(81)	Flask 1 R ..	1·0	,,	Turbid in 24 hours. Plates poured 14.6.1893.
(82)	,, ..	0·5	,,	Turbid in 24 hours.
		Typhoid-infected unsterilised Thames + 3 *per cent.* NaCl.		
(77)	Flask 1 I ..	1·0	3 drops	Turbid in 48 hours. Plates poured 15.6.1893.
(78)	,, ..	0·5	,,	Turbid in 48 hours.
(83)	Flask 1 R ..	1·0	,,	Turbid in 48 hours.
(84)	,, ..	0·5	,,	Turbid in 48 hours. Plates poured 15.6.1893.

The six turbid broth tubes, indicated above as selected for further examination by gelatine plate culture, yielded the following results :—

Broth tube.

(73.) *Typhoid-infected Unsterilised Thames* + 0·1 *per cent.* NaCl, *Flask* 1 *Incubator.* (*Typhoid Absent.*)

The colonies yielded pink growths on potatoes and liquefied gelatine; they were therefore not those of typhoid.

(79.) *Typhoid-infected Unsterilised Thames* + 0·1 *per cent.* NaCl, *Flask* 1 *Refrigerator.* (*Typhoid Absent.*)

The colonies presented some resemblance to typhoid, as did also the growths on potatoes obtained from them. No indol reaction. On inoculating into gelatine tubes, the latter were found to very slowly liquefy on long keeping. On inoculating

from such a liquefied tube on to potatoes, the same typhoid-like growth was obtained. This organism, which was again subsequently met with, might easily lead to a false diagnosis of typhoid, unless the gelatine tubes were preserved for some time.

Broth tube.

(75.) *Typhoid-infected Unsterilised Thames* + 1 *per cent.* NaCl, *Flask* 1 *Incubator.* (*Typhoid Absent.*)

The colonies on transference to potatoes gave rise to light-brown growth, not like typhoid.

(82.) *Typhoid-infected Unsterilised Thames* + 1 *per cent.* NaCl, *Flask* 1 *Refrigerator.* (*Typhoid Absent.*)

The colonies liquefied the gelatine, and gave rise to easily visible growths on potatoes.

(78.) *Typhoid-infected Unsterilised Thames* + 3 *per cent.* NaCl, *Flask* 1 *Incubator.* (*Typhoid Absent.*)

The colonies gave rise to pink growths on potatoes; therefore certainly not typhoid.

(84.) *Typhoid-infected Unsterilised Thames* + 3 *per cent.* NaCl, *Flask* 1 *Refrigerator.* (*Typhoid Absent.*)

Same results as with No. 78 above.

Thus in the case of none of these saline waters could a diagnosis of typhoid bacilli be made on 13.6.1893; *by reference to p.* 433 *it will be seen also that on* 14.6.1893 *the typhoid bacilli were no longer demonstrable in the unsterilised Thames water to which no salt had been added.*

These saline waters were again examined by phenol broth-culture on 21.6.1893, with the following results:—

Examination by Phenol Broth-culture, 21.6.1893.

Number of broth tube.	Water used for cultivation with phenol broth.	Quantity of water taken in c.c.	Quantity of phenol solution added to 10 c.c. broth.	Remarks.
		Typhoid-infected Unsterilised Thames + 0·1 *per cent.* NaCl.		
(88)	Flask 1 I ..	1·0	3 drops	Turbid in 48 hours. Plates poured 23.6.1893.
(91)	„ 1 R..	1·0	„	„ „ „ „
		Typhoid-infected Unsterilised Thames + 1·0 *per cent.* NaCl.		
(89)	Flask 1 I ..	1·0	3 drops	Turbid in 48 hours. Plates poured 23.6.1893.
(92)	„ 1 R..	1·0	„	„ „ „ „
		Typhoid-infected Unsterilised Thames + 3·0 *per cent.* NaCl.		
(90)	Flask 1 I ..	1·0	3 drops	Turbid in 48 hours. Plates poured 23.6.1893.
(93)	„ 1 R..	1·0	„	„ „ „ „

Thus, on this occasion, all the waters reacted in forty-eight hours with the phenol broth-solution. The following results were obtained on plate cultivating the turbid broth tubes :—

Broth tube.

(88.) *Typhoid-infected Unsterilised Thames* + 0·1 *per cent.* NaCl, *Flask* 1 *Incubator.* (*Typhoid Absent.*)

The colonies on transference to potatoes yielded light brown growths, which were certainly not due to typhoid.

(91.) *Typhoid-infected Unsterilised Thames* + 0·1 *per cent.* NaCl, *Flask* 1 *Refrigerator.* (*Typhoid Absent.*)

The potato growths from colonies somewhat resembled typhoid; there was also no indol reaction, but gelatine tubes were slowly liquefied by the organism, which was, therefore, not typhoid. (See similar experiences with No. 79, p. 443.)

(89.) *Typhoid-infected Unsterilised Thames* + 1 *per cent.* NaCl, *Flask* 1 *Incubator.* (*Typhoid Absent.*)

The potato growths from colonies were thick and brown in colour, quite unlike those of typhoid.

Broth tube.

(92.) *Typhoid-infected Unsterilised Thames* + 1 *per cent.* NaCl, *Flask* 1 *Refrigerator.* (*Typhoid Absent.*)

The potato growths from colonies were pink in colour, and, on inoculation into gelatine, liquefied it. Certainly not typhoid.

(90.) *Typhoid-infected Unsterilised Thames* + 3 *per cent.* NaCl, *Flask* 1 *Incubator.* (*Typhoid Absent.*)

The potato growths from colonies were pinkish-white in colour and much too conspicuous for typhoid.

(93.) *Typhoid-infected Unsterilised Thames* + 3 *per cent.* NaCl, *Flask* 1 *Refrigerator.* (*Typhoid Absent.*)

The potato growths from colonies were of a dirty white colour, and on inoculation into gelatine the latter was liquefied. Therefore, certainly not typhoid.

Thus, again, on this occasion, 21.6.1893, *it was found impossible to demonstrate the presence of typhoid bacilli in any of these saline waters.*

The saline waters were again examined by phenol broth-culture on 26.6.1893, with the following results:—

Examination by Phenol Broth-culture, 26.6.1893.

Number of broth tube.	Water used for cultivation with phenol broth.	Quantity of water taken in c.c.	Quantity of phenol solution added to 10 c.c. broth.	Remarks.
	Typhoid-infected Unsterilised Thames + 0·1 *per cent.* NaCl.			
(98)	Flask 1 I ..	1·0	3 drops	Turbid in 48 hours.
(101)	„ 1 R..	„	„	„ „
	Typhoid-infected Unsterilised Thames + 1·0 *per cent.* NaCl.			
(99)	Flask 1 I ..	1·0	3 drops	Turbid in 48 hours.
(102)	„ 1 R..	„	„	„ „
	Typhoid-infected Unsterilised Thames + 3·0 *per cent.* NaCl.			
(100)	Flask 1 I ..	1·0	3 drops	Turbid in 48 hours.
(103)	„ 1 R..	„	„	„ „

These turbid broth tubes were submitted to plate cultivation on 28.6.1893, and the following results obtained:—

Broth tube.

(98.) *Typhoid-infected Unsterilised Thames* + 0·1 *per cent.* NaCl, *Flask* 1 *Incubator.* (*Typhoid Absent.*)

The plates exhibited liquefying and small colonies respectively. From the small colonies, potatoes were inoculated, a brown and slimy growth being obtained, on inoculation from which into gelatine tubes, a blue, fluorescent, non-liquefying growth resulted. Thus nothing like typhoid was present.

(101.) *Typhoid-infected Unsterilised Thames* + 0·1 *per cent.* NaCl, *Flask* 1 *Refrigerator.* (*Typhoid Absent.*)

The potato-growths obtained from the colonies bore some resemblance to typhoid, although the surface was rather too shining. On inoculating into gelatine tubes, it was found that very slow liquefaction took place. This was, therefore, the same organism which had been several times before met with in this flask. Under the microscope the bacilli also present some resemblance to typhoid, but they have squarer ends.

(99.) *Typhoid-infected Unsterilised Thames* + 1 *per cent.* NaCl, *Flask* 1 *Incubator.* (*Typhoid Absent.*)

On the plates there were small colonies forming surface expansions with green fluorescence ; these gave rise, on potatoes, to thick, greyish-brown growths quite unlike typhoid.

(102.) *Typhoid-infected Unsterilised Thames* + 1 *per cent.* NaCl, *Flask* 1 *Refrigerator.* (*Typhoid Absent.*)

The plates contained both liquefying and small colonies, the latter, on transferring to potatoes, gave strong, conspicuous, and flesh-coloured growths quite unlike typhoid.

(100.) *Typhoid-infected Unsterilised Thames* + 3 *per cent.* NaCl, *Flask* 1 *Incubator.* (*Typhoid Absent.*)

The plates contained small colonies forming surface expansions, which gave light brown growths on potatoes quite unlike typhoid.

(103.) *Typhoid-infected Unsterilised Thames* + 3 *per cent.* NaCl, *Flask* 1 *Refrigerator.* (*Typhoid Absent.*)

The plates contained liquefying and small colonies respectively, the potato-growths from the latter were strong, thick, waxy, and greyish-white, quite unlike typhoid.

Thus, on this occasion (26.6.1893) *again, the presence of typhoid bacilli could not be demonstrated in any of these saline waters.*

The final examination of these saline waters was made on 5.7.1893, with the following results:—

Examination by Phenol Broth-culture on 5.7.1893.

Number of broth tube.	Water used for cultivation with phenol broth.	Quantity of water taken. c.c.	Quantity of phenol solution added to 10 c.c. broth.	Remarks.
		Typhoid-infected Unsterilised Thames + 0·1 *per cent.* NaCl.		
(113)	Flask 1 I ..	1·0	3 drops	Turbid in 48 hours.
(123)	" ..	"	5 "	Not turbid in 6 days.
(116)	Flask 1 R ..	"	3 "	Turbid in 48 hours.
(126)	" ..	"	5 "	Turbid in 6 days. Plates poured.
		Typhoid-infected Unsterilised Thames + 1 *per cent.* NaCl.		
(114)	Flask 1 I ..	1·0	3 drops	Turbid in 48 hours.
(124)	" ..	"	5 "	Not turbid in 6 days.
(117)	Flask 1 R ..	"	3 "	Turbid in 24 hours. Plates poured 6.7.1893.
(127)	" ..	"	5 "	Not turbid in 6 days.
		Typhoid-infected Unsterilised Thames + 3 *per cent.* NaCl.		
(115)	Flask 1 I ..	1·0	3 drops	Turbid in 72 hours.
(125)	" ..	"	5 "	Not turbid in 6 days.
(118)	Flask 1 R ..	"	3 "	Turbid in 48 hours.
(128)	" ..	"	5 "	Not turbid in 6 days.

Plate cultivations were made of the turbid broth tubes with the following results :—

Broth tube.

(113.) *Typhoid-infected Unsterilised Thames* + 0·1 *per cent.* NaCl, *Flask* 1 *Incubator*. (*Typhoid Absent.*)

The plates had the appearance of being pure cultivations of an organism producing fluorescent expansion colonies without liquefaction. The colonies in which fluorescence was least conspicuous and which had, therefore, most chance of being typhoid, were transferred to potatoes, on which they yielded a greyish-brown growth sharply distinguishable from the potato and quite unlike typhoid.

(116.) *Typhoid-infected Unsterilised Thames* + 0·1 *per cent.* NaCl, *Flask* 1 *Refrigerator*. (*Typhoid Absent.*)

The plates exhibited a number of small colonies giving rise to surface expansions something like those of typhoid. These

colonies, on being transferred to potatoes, again yielded inconspicuous colourless growths very similar to those of typhoid. Negative results were also obtained with the milk, indol, and gas-bubble reactions. It was found, however, that the gelatine-tubes inoculated from these typhoid-like colonies underwent very slow liquefaction. This was, therefore, obviously the same organism which had been repeatedly obtained before from this flask, and the superficial resemblance to typhoid of which has been already referred to (see pp. 445, 447).

Broth tube.
(114.) *Typhoid-infected Unsterilised Thames* + 1 *per cent.* NaCl, *Flask* 1 *Incubator.* (*Typhoid Absent.*)

The plates contained fluorescent non-liquefying colonies. The less obviously fluorescent colonies on transference to potatoes yielded growths which were not sufficiently different from typhoid to decide, whilst the milk, indol, and gas-bubble tests were also negative. On inoculation into gelatine tubes, however, the latter became fluorescent.

(117.) *Typhoid-infected Unsterilised Thames* + 1 *per cent.* NaCl, *Flask* 1 *Refrigerator.* (*Typhoid Absent.*)

The plates exhibited a pure cultivation of bacillus, giving rise to small, cup-shaped, liquid colonies, probably *B. liquidus* (Percy Frankland), which is very frequently found to survive in the 3-drop phenol broth-cultures.

(115.) *Typhoid-infected Unsterilised Thames* + 3 *per cent.* NaCl, *Flask* 1 *Incubator.* (*Typhoid Absent.*)

The plates exhibited small, milk-drop colonies, with slight tendency to expand. Microscopic examination showed them to be due to bacilli thinner than the typhoid bacillus, and on potatoes they yielded light yellow, shining growths, unlike those of typhoid.

(118.) *Typhoid-infected Unsterilised Thames* + 3 *per cent.* NaCl, *Flask* 1 *Refrigerator.* (*Typhoid Absent.*)

The plates contained a number of liquefying colonies, apparently *B. liquidus* (Percy Frankland), also some small colonies, some of which gave rise to very small surface expansions and very small milk-drops. The potatoes inoculated from the milk-drop colonies yielded flesh-coloured growths, whilst those from the expansion colonies were not decisive.

Plates were again poured from these potatoes, and very small surface-expansion colonies again obtained, which, however, again were not like those of typhoid, and gelatine tubes inoculated from these yielded, in course of time, brown surface growths quite unlike typhoid.

The results of these experiments with the unsterilised Thames water, to which 0·1, 1, and 3 per cent. of common salt respectively had been added, are instructive in more ways than one. Thus:—

(1.) They show that the addition of the salt stimulated the growth and multiplication of some of the water bacteria to an enormous extent, the effect being the most marked with the largest proportion of salt (3 per cent.), whilst the water to which only 0·1 per cent. of salt was added, behaved almost exactly like the untreated Thames water.

(2.) This multiplication was, as usual, followed by decline, but the saline waters remained, even after six weeks, more densely, and with the larger proportion of salt much more densely, populated than the Thames water to which no salt was added.

(3.) As regards the effect of the salt addition on the typhoid bacilli present in the water, the experiments show that they were most prejudicially influenced. Thus whilst on the eighteenth day after infection the typhoid bacilli were easily demonstrable in the ordinary unsterilised Thames water to which no salt had been added, and also in that which had received 0·1 per cent. of salt and which had been kept at 6—8° C., they were not discoverable in any of the waters to which 1 and 3 per cent. salt had been added, nor in that which had received only 0·1 per cent. salt, but which had been kept at the summer temperature of 19° C.

(4.) In the case of the 3 per cent. salt addition, I am of opinion that the rapid disappearance of the typhoid bacilli is largely due to the direct action of the salt, whilst in the case of the smaller proportions it may also be due to the great multiplication of some of the common water bacteria.

Further experiments on the behaviour of typhoid bacilli in Thames water, to which salt had been added, were subsequently made (see pp. 530, *et seq.*), they proved entirely confirmatory of the results just recorded above, both as to the stimulation of the multiplication of the

water bacteria, and as to the more rapid disappearance of the typhoid bacilli. The directly prejudicial action of the salt on the typhoid bacillus was further demonstrated by the addition of salt to steam-sterilised Thames water containing typhoid bacilli.

Behaviour of the Typhoid Bacillus and of the B. coli communis *in Steam-sterilised Thames Water (First Series of Experiments).*

It will now be interesting to consider the behaviour of these bacilli in the precisely parallel series of experiments made with the same sample of Thames water which had been previously sterilised by steam. These experiments are of importance more especially because they enable us to ascertain whether the water contains the necessary food materials for these particular bacteria, as, owing to the absence of other forms, it is now possible to determine how the actual numbers of these bacteria are affected by residence in the water.

The infection and distribution of these steam-sterilised waters has already been described on pp. 410 and 411, so that I can pass at once to their subsequent examination, made both by gelatine plate and phenol broth-culture, at different intervals of time.

In the table (p. 452) the fate of the typhoid bacilli introduced into the steam-sterilised Thames water on 11.5.1893, is followed over a period of seventy-six days, and it will be seen that during this time their numbers underwent an almost continuous decline. Thus, whilst at the outset they were present to the number of, in round numbers, 70,000 per cub. cm., at the end of this period their presence was only just demonstrable by gelatine plate cultivation at all, and not more than from 6—12 were discoverable in 1 cub. cm. The most important feature in this chronicle of their deportment is the circumstance that they exhibited no multiplication or increase in numbers during their residence in the water, clearly showing, therefore, that the latter did not afford the nutriment and other conditions necessary for the proliferation of the typhoid bacilli. In fact, the decline from the commencement is an unbroken one, with the exception of the solitary observation of an increase from 27,000 on 22.5.1893, to 42,000 on 29.5.1893, in the case of the water maintained at a winter temperature. Whatever may have been the cause of this increase, it is not sufficiently great to be comparable with that extensive reproduction which takes place in the case of those bacteria which are the natural inhabitants of water. Moreover, that the water was not suited to the well-being of the typhoid bacilli was further testified to by the fact that the colonies on the gelatine plates became smaller, feebler, and more degenerate as time went on, until on the occasion of the last few examinations, they were, with difficulty, recognisable as typhoid colonies, and exhibited a marked disinclination to form the characteristic growths expanding over the surface of the gelatine.

Behaviour of Typhoid Bacillus in Steam-sterilised Thames Water, infected 11.5.1893.

Dates on which plate cultivations were made.	Particular flask employed.		Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies obtained from 1 c.c. of water.	
	Incubator.	Refrigerator.			Incubator flasks.	Refrigerator flasks.
11.5.1893	Before subdivision		4	c.c. $\frac{1}{2}$ and $\frac{1}{5}$	74,000	
16.5.1893	1 I		3	$\frac{1}{5}$ and $\frac{1}{10}$	48,000	
,,		1 R	3	$\frac{2}{11}$ and $\frac{1}{11}$		51,000
22.5.1893	1 I		4	$\frac{1}{4}$ and $\frac{1}{12}$	27,000	
,,		1 R	3	1·0 and 0·5		27,000
29.5.1893	1 I		4	$\frac{3}{10}$ and $\frac{1}{10}$	24,000	
,,		1 R	4	$\frac{1}{5}$ and $\frac{1}{10}$		42,000
5.6.1893	1 I		4	$\frac{1}{5}$ and $\frac{1}{10}$	15,000	
,,		1 R	4	$\frac{1}{6}$ and $\frac{1}{12}$		40,000
6.7.1893	1 I		6	$\frac{1}{6}$ and $\frac{1}{12}$	5,000	
,,		1 R	8	$\frac{1}{5}$ and $\frac{1}{10}$		275
18.7.1893	1 I		8	$\frac{1}{2}$ and $\frac{1}{6}$	Only a few typhoid colonies.	
,,		1 R	8	$\frac{2}{3}$ and $\frac{1}{3}$		0
25.7.1893	1 I		4	1·0 and $\frac{3}{10}$	Only a few typhoid colonies.	
,,		1 R	6	1·0 and $\frac{3}{10}$	Only a few depth colonies, appeared to be typhoid.	
26.7.1893		1 R	5	3 and 4	Only 1 small depth colony, probably typhoid in a very degenerated state.	

As regards the effect of the higher or lower temperature at which the waters were maintained, it appeared throughout the greater part of the time that the typhoid bacilli in the flask, kept at the summer temperature (19° C.), suffered more rapid degeneration than those in the water, which was preserved at the winter temperature of 6° C., although quite at the last this relationship was reversed.

It is interesting to contrast, with the above results, the behaviour of the *B. coli communis* placed under precisely similar conditions in the same water and over the same period of time. The results of this comparative investigation are recorded in the following table :—

Behaviour of *Bacillus Coli communis* in Steam-sterilised Thames water, infected 11.5.1893.

Dates on which plate cultivations were made.	Particular flask employed.		Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies obtained from 1 c.c. of water.	
	Incubator.	Refrigerator.			Incubator flasks.	Refrigerator flasks.
11.5.1893	Before subdivision		4	c.c. $\frac{5}{9}$ and $\frac{2}{9}$	69,000	
16.5.1893	1 I		3	$\frac{1}{6}$ and $\frac{1}{12}$	643,000	
,,		1 R	3	$\frac{1}{50}$ and $\frac{1}{10}$		107,000
23.5.1893	1 I		3	$\frac{1}{5}$ and $\frac{1}{10}$	224,000	
,,		1 R	3	$\frac{1}{5}$ and $\frac{1}{10}$		78,000
30.5.1893	1 I		3	$\frac{1}{6}$ and $\frac{1}{12}$	281,000	
,,		1 R	3	$\frac{1}{6}$ and $\frac{1}{12}$		101,000
5.6.1893	1 I		4	$\frac{1}{6}$ and $\frac{1}{12}$	192,000	
,,		1 R	4	$\frac{2}{11}$ and $\frac{1}{11}$		97,000
6.7.1893	1 I		6	$\frac{1}{6}$ and $\frac{1}{12}$	117,000	
,,		1 R	6	$\frac{2}{9}$ and $\frac{1}{9}$		28,000
18.7.1893	1 I		4	$\frac{1}{5}$ and $\frac{1}{10}$	1,500	
,,		1 R	4	$\frac{1}{5}$ and $\frac{1}{10}$		4,000
25.7.1893	1 I		4	$\frac{8}{9}$ and $\frac{2}{3}$	Coli colonies still abundantly present, but exact estimation difficult in consequence of air contamination of these plates.	
,,		1 R	4	$\frac{1}{5}$ and $\frac{1}{10}$		5,000 (not contaminated)

From the above table it will be seen that the *B. coli communis* presents a great contrast to the typhoid bacillus in respect of its behaviour in this steam-sterilised Thames water. Thus, at the commencement of the experiment, both bacilli were present in about the same numbers, the typhoid-infected water containing 74,000, and the coli-infected water, 69,000 bacilli per 1 c.c. respectively, but whilst the subsequent numbers found in the typhoid-infected waters were invariably less than this initial number in the case of the coli-infected waters, the initial number was subsequently very greatly exceeded, the observed multiplication being greatest in the water kept at a summer temperature. This multiplication was afterwards followed by a corresponding decline, but even at the end of the seventy-five days over which the observations were extended, the number of the coli bacilli greatly exceeded that of the typhoid bacilli. It is, moreover, worthy of remark that the coli-infected water kept at 19° C., in which the most extensive multiplication took place, ultimately contained a smaller number of coli bacilli than the water maintained at 6° C., in which the multiplication had been less considerable.

The above results, obtained by the gelatine plate cultivation of the typhoid and coli-infected steam-sterilised Thames waters, were repeatedly confirmed and supplemented by the method of phenol broth-culture, thus :—

Examination by Phenol Broth-culture on 5.6.1893.

Number of broth tube.	Water used for cultivation with phenol broth.	Quantity of water taken in c.c.	Quantity of phenol solution added to 10 c.c. broth.	Remarks.
				Typhoid-infected Steam-sterilised Thames.
(53)	Flask 1 I ..	0·5	3 drops	Turbid in 24 hours.
(54)	,, ..	1·0	,,	,, ,,
(55)	Flask 1 R ..	0·5	,,	,, ,,
(56)	,, ..	1·0	,,	,, ,,
(61)	Flask 1 I ..	0·5	5 drops	Turbid in 24 hours. Plates poured 6.6.1893.
(62)	,, ..	1·0	,,	Turbid in 24 hours.
(63)	Flask 1 R ..	0·5	,,	Very slight turbidity in 24 hours, pronounced turbidity in 48 hours.
(64)	,, ..	1·0	,,	Turbid in 24 hours.
				Coli-infected Steam-sterilised Thames.
(49)	Flask 1 I ..	0·5	3 drops	Turbid in 24 hours.
(50)	,, ..	1·0	,,	,, ,,
(51)	Flask 1 R ..	0·5	,,	,, ,,
(52)	,, ..	1·0	,,	,, ,,
(57)	Flask 1 I ..	0·5	5 drops	Turbid in 24 hours. Plates poured 6.6.1893.
(58)	,, ..	1·0	,,	Turbid in 24 hours.
(59)	Flask 1 R ..	0·5	,,	Turbid in 24 hours. Plates poured 6.6.1893.
(60)	,, ..	1·0	,,	Turbid in 24 hours.

These examinations by phenol broth-culture show, therefore, that, on 5.6.1893, the typhoid-infected steam-sterilised Thames water reacted already in twenty-four hours with the test, irrespectively of whether 3 drops or 5 drops of phenol solution were added to the 10 c.c. of broth; whilst, by referring back to p. 427, it will be seen that the typhoid-infected unsterilised Thames water only reacted in twenty-four hours, even with the 3 drops of phenol solution, in the case of the water which had been kept at the winter temperature, whilst the summer temperature water only reacted after forty-eight hours. From these comparative tests it can be inferred, therefore, that, on the date in question, the typhoid bacilli were in a less vigorous condition in the unsterilised than in the sterilised water.

Of the plate cultivations made from the turbid broth tubes Nos. 61, 57, and 59, those from No. 61 yielded typical typhoid colonies which were confirmed by growth on potatoes and by negative results with the indol and gas-bubble tests; the plates from Nos. 57 and 59,

on the other hand, yielded the typical colonies of the *B. coli communis*, and these were further confirmed by growth in potatoes, and by positive results with the indol and gas-bubble tests.

The above phenol broth-culture tests were all made with 0·5 and 1·0 c.c. of the water, but on the same day (5.6.1893) some further experiments were made to incidentally determine whether much smaller volumes (a single drop) of water would react, and, if so, whether with equal rapidity. Thus—

Examination by Phenol Broth-culture of Small Quantities of Infected Water, 5.6.1893.

Number of broth tube.	Water used for cultivation with phenol broth.	Quantity of water taken in c.c.	Quantity of phenol solution added to 10 c.c. broth.	Remarks.
		Typhoid-infected Steam-sterilised Thames.		
(65)	Flask 1 I ..	1 drop	3 drops	Turbid in 24 hours.
(66)	„ ..	„	5 „	Very slightly turbid in 24 hours; turbid in 48 hours.
(67)	Flask 1 R ..	„	3 „	Turbid in 24 hours.
(68)	„ ..	„	5 „	Not turbid in 24 hours; turbid in 48 hours.
		Coli-infected Steam-sterilised Thames.		
(69)	Flask 1 I ..	1 drop	3 drops	Turbid in 24 hours.
(70)	„ ..	„	5 „	„ „
(71)	Flask 1 R ..	„	3 „	„ „
(72)	„ ..	„	5 „	„ „

The interest attaching to the phenol broth examinations consists in the circumstance that the actual number of typhoid and coli bacilli present in the volumes of water used can be calculated from the results of the plate cultivations made on the same day (see pp. 452 and 454). Thus, it will be seen from the tables on pp. 456 and 457, that there was, in nearly all cases, practically no difference in the time which elapsed before the phenol broth tubes became turbid, irrespectively of whether 0·5 c.c., 1 c.c., or only 1 drop of the same water was employed; for, even in the 1 drop of the water, it is apparent from the plate cultivations (p. 452) that there must have been upwards of 1000 typhoid bacilli present, and a still larger number of coli bacilli in those waters infected with this bacillus.

Another examination by phenol broth-culture was made of the typhoid-infected steam-sterilised Thames water about one month later, on 5.7.1893 and on 6.7.1893, and for the last time on 25.7.1893. Thus—

Examination by Phenol Broth-culture.

Number of broth tube.	Water used for cultivation with phenol broth.	Quantity of water taken in c.c.	Quantity of phenol solution added to 10 c.c. broth.	Remarks.
		Typhoid-infected Steam-sterilised Thames.		
5.7.1893				
(110)	Flask 1 I ..	1 drop	3 drops	Turbid in 24 hours.
(112)	,, ..	,,	5 ,,	,, 48 ,,
6.7.1893				
(129)	Flask 1 R ..	1 drop	3 drops	Remained clear.
(130)	,, ..	,,	5 ,,	,, ,,
(131)	,, ..	0·5 c.c.	3 ,,	Turbid in 72 hours.
(132)	,, ..	,,	5 ,,	,, ,,
25.7.1893				
(199)	Flask 1 I ..	1·0 c.c.	3 drops	Turbid in 48 hours.
(200)	,, 1 R ..	,,	3 ,,	,, ,,
		Coli-infected Steam-sterilised Thames.		
(195)	Flask 1 I ..	1·0 c.c.	3 drops	Turbid in 48 hours.
(196)	,, 1 R ..	,,	,,	,, ,,

From the above it will be seen that even on 25.7.1893, when the plate cultivations (see p. 452) were only yielding about twelve colonies per c.c., and these colonies of a very feeble and degenerate character, the phenol broth-cultures of 1 c.c. of the water still became turbid in forty-eight hours, and thus revealed the presence of living typhoid bacilli with the greatest facility.

On the other hand, when the number of typhoid bacilli in the water is small, it may very easily happen that a phenol broth tube now and again may fail to go turbid (as in the case of Broth tubes 443 and 444, see table above), and it is very necessary, therefore, to exercise great caution, and not to draw conclusions from a single observation, but only after a number of repeated trials.

The examinations by phenol broth-culture of these infected steam-sterilised Thames waters thus entirely substantiate the results arrived at

by the direct method of plate cultivation, and show that both the typhoid and coli bacilli were still present in a living state in this water, irrespectively of whether it had been preserved at a summer or a winter temperature, for a period of seventy-five days.

Behaviour of the Typhoid Bacillus and of the B. coli communis *in Thames Water sterilised by Filtration through Porous Porcelain. (First Series of Experiments.)*

The preparation and infection of this water has already been described (see pp. 410 and 411), and, as already indicated, the infected water was placed under precisely the same conditions of temperature, &c., as the steam-sterilised and unsterilised waters. In the periodical examination of this water the following results were obtained :—

Behaviour of Typhoid Bacillus in Thames Water sterilised by Filtration through Porous Porcelain, infected 11.5.1893.

Dates on which plate cultivations were made.	Particular flask employed.		Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies obtained from 1 c.c. of water.	
	Incubator.	Refrigerator.			Incubator flask.	Refrigerator flasks.
11.5.1893	Before subdivision.		4	c.c. $\frac{1}{2}$ and $\frac{1}{5}$	75,000	
16.5.1893	1 I		9	$\frac{1}{6}$ and $\frac{1}{12}$	0 (plates quite clear)	
		1 R	6	$\frac{1}{5}$ and $\frac{1}{10}$		6700
22.5.1893	1 I		8	1·5 and 0·5	0	
		1 R	8	$\frac{3}{10}$ and $\frac{1}{10}$		0
29.5.1893	1 I		12	1·5 and 0·5	0	
		1 R	12	$\frac{5}{6}$ and $\frac{5}{12}$		0

The above results were most unexpected, for they show that, although as many as 75,000 typhoid bacilli per 1 c.c. were introduced into this water, they were entirely destroyed in five days at 19° C., and had undergone a very large reduction in number at 6—8° C., whilst in twelve days they were no longer discoverable in this water kept at the low temperature. On 2.6.1893 sterile broth was added to the flasks, which were then placed in the incubator at 38° C., but even this treatment did not lead to any revivification of the typhoid bacilli, which could neither be detected by plate cultivation nor phenol broth-culture.

The same rapid destruction of the *B. coli communis* was observed in this water, as will be seen from the following table :—

Behaviour of *B. coli communis* in Thames Water sterilised by Filtration through Porous Porcelain, infected 11.5.1893.

Dates on which plate cultivations were made.	Particular flask employed.		Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies obtained from 1 c.c. of water.	
	Incubator.	Refrigerator.			Incubator flask.	Refrigerator flask.
11.5.1893	Before subdivision.		4	c.c. $\frac{1}{2}$ and $\frac{1}{5}$	83,000	
16.5.1893	1 I		9	$\frac{1}{5}$ and $\frac{1}{10}$	0	
		1 R	6	$\frac{1}{6}$ and $\frac{1}{12}$		8600
23.5.1893	1 I		7	$1\frac{4}{11}$ and $\frac{5}{11}$	0	
		1 R	7	$\frac{1}{3}$ and $\frac{1}{6}$		0
30.5.1893	1 I		11	1·5 and 0·5	0	
		1 R	11	$1\frac{4}{11}$ and $\frac{5}{11}$		0

Thus, in the case of the *B. coli communis* again, there was the same disappearance in five days of the bacilli in the water kept at 19° C., the great diminution in numbers in the same time in the water kept at 6—8° C., followed by complete disappearance of the bacilli in this water also by the twelfth day. Similar attempts made by the addition of sterile broth to resuscitate the bacilli in these waters also proved unavailing.

These results, showing that the typhoid and coli bacilli were more rapidly destroyed in the porcelain-filtered than in the unsterilised, and far more rapidly than in the steam-sterilised, Thames water, were so surprising that it was necessary to banish every suspicion of some accidental disturbing cause having arisen in these experiments.

The most obvious suggestion was that the filter itself might have introduced some antiseptic substance into the water. This was, however, highly improbable, as the filter in question had only been previously used for the similar sterilisation of Thames and Loch Katrine waters. In order to abolish this objection, however, the porcelain cylinder was thoroughly scrubbed externally with a tooth-brush, and then upwards of 30 litres of distilled water passed through it. The filter was then steam sterilised and employed for the filtration of some more of the same Thames water, which was infected with typhoid and coli as below. Thus—

(*a.*) *Typhoid Bacillus.*—20 needle-loops were taken from an agar cultivation of the typhoid bacillus of nine days age and introduced into 50 c.c. of steam-sterilised water; after thoroughly shaking, 3 c.c. of this water-attenuation were added to 750 c.c. of the porcelain-filtered Thames water.

(*b.*) *Bacillus coli communis.*—The infection was made in exactly the same way in every detail, the agar culture being also of the same age.

The waters thus infected with typhoid and coli respectively were subdivided into smaller flasks, some of which were placed as usual in the incubator at 19° C. and others in the refrigerator at 6—8° C. The following results were obtained on examination :—

Behaviour of the Typhoid Bacillus and *B. coli communis* in Thames Water Sterilised by Filtration through Porous Porcelain. Infected 15.6.1893.

Dates on which plate cultivations were made.	Particular flask employed.		Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies obtained from 1 c.c. of water.	
	Incubator.	Refrigerator.			Incubator flask.	Refrigerator flask.
Typhoid Bacillus.						
15.6.1893	Before subdivision.		3	c.c. $\frac{1}{8}$ and $\frac{1}{16}$	66,000	
20.6.1893	1 I		8	$\frac{1}{5}$ and $\frac{1}{10}$	0	
		1 R	8	$\frac{1}{6}$ and $\frac{1}{12}$		84
B. coli communis.						
15.6.1893	Before subdivision.		3	c.c. $\frac{2}{13}$ and $\frac{1}{13}$	125,000	
20.6.1893	1 I		8	$\frac{1}{5}$ and $\frac{1}{10}$	0	
		1 R	8	$\frac{1}{6}$ and $\frac{1}{12}$		0

These results, therefore, entirely confirm those previously obtained, the disappearance of both the typhoid and coli bacilli being even still more rapid than on the former occasion.

Results of a similar character were also subsequently obtained with other waters sterilised by filtration (see pp. 479, 483, 502), in some of which, moreover, totally different filters, constructed of infusorial earth, were employed.

Second Series of Experiments.

The Behaviour of the Typhoid Bacillus and of the B. coli communis *in Loch Katrine Water.*

Having, in the first series of experiments, determined the behaviour of these bacilli in a typical calcareous surface water like that of the Thames, which receives the drainage from cultivated land, I proceeded in the next instance to carry out a somewhat similar series o experiments with Loch Katrine water, which may be taken as a type of an upland surface water derived almost exclusively from uncultivated land, and of a somewhat peaty character.

The sample of Loch Katrine was collected from a tap on the main in the Broomielaw, Glasgow, on June 30th, 1893.

Submitted to plate cultivation on the spot, it was found to contain 112 bacteria in 1 c.c., whilst on chemical analysis it yielded the following figures:—

Results of Analysis expressed in Parts per 100,000.

	Loch Katrine water uninfected.	Loch Katrine water infected with typhoid.	Loch Katrine water infected with *B. coli.*
Total solid matter	2·60		
Organic carbon } by combustion	0·185		
Organic nitrogen } by combustion	0·019		
Organic nitrogen (by Kjeldahl process)	0·013		
Ammonia (free)	0	0	
" (albuminoid)	0·006	0·013	
Oxygen consumed by organic matter	0·144	0·151	0·140
Nitrogen as nitrates and nitrites	0·006		
Total combined nitrogen	0·025		
Chlorine	0·65	0·65	0·65
Temporary hardness	0		
Permanent "	0·8		
Total "	0·8		
Proportion of organic carbon to organic nitrogen in suspended organic matter	8·14 : 1		

Infection of Loch Katrine Water with Typhoid and B. coli communis, 4.7.1893.

The cultures of the bacilli employed were on agar, and in both cases twenty-eight days old. In the case of the typhoid bacillus 40 needle-loops, and in that of the coli 25 loops, were taken from the surface of the agar, removing as little of the culture-material as possible, and introduced in each case into 50 c.c. of steam-sterilised water, which was then violently shaken to ensure disintegration of the bacterial masses. The experimental waters were then infected from these water-attenuations as follows :—

	Typhoid bacillus.	*Bacillus coli communis.*
Unsterilised Loch Katrine water	2,000 c.c. infected with 8 c.c. of water attenuation	1,000 c.c. infected with 3 c.c. of water attenuation.
Steam-sterilised Loch Katrine water	750 c.c. infected with 3 c.c. of water attenuation	750 c.c. infected with 2 c.c. of water attenuation.
Porcelain-filtered Loch Katrine water	750 c.c. infected with 3 c.c. of water attenuation	750 c.c. infected with 2 c.c. of water attenuation.

These infected waters, after thorough agitation, were then, as in previous experiments, subdivided amongst a number of small sterile conical flasks, plugged with sterile cotton-wool; in each case some of these flasks were placed in the incubator at 19° C., whilst others were kept in a refrigerator at 6—8° C. The uninfected unsterilised Loch Katrine water was also put into similar flasks, which were kept under precisely similar conditions for control.

1. *Bacteriological Examination of the Unsterilised Uninfected Loch Katrine Water.*

The control-waters were submitted to periodical examination both by gelatine-plate and phenol-broth culture, with the following results :—

Uninfected Unsterilised Loch Katrine Water. (First Series)

(Date of collection, June 30, 1893.)

Dates on which plate cultivations were made.	Particular flask employed.		Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies obtained from 1 c.c. of water.	
	Incubator.	Refrigerator.			Incubator flask.	Refrigerator flask.
4.7.1893	Before subdivision.		3—4	c c. $\frac{1}{30}$ and $\frac{1}{60}$	121 (Only few liquefying colonies.)	
10.7.1893	1 I		3—4	$\frac{1}{6}$, $\frac{1}{12}$, $\frac{1}{30}$, and $\frac{1}{60}$	925 (Only few liquefying colonies.)	
		1 R	3—5	$\frac{1}{6}$, $\frac{1}{12}$, $\frac{1}{30}$, and $\frac{1}{60}$		1150 (Only few liquefying colonies.)
17.7.1893	1 I		3—5	$\frac{1}{5}$, $\frac{1}{10}$, $\frac{1}{25}$, and $\frac{1}{50}$	716 (Few liquefying colonies.)	
		1 R	2—3	$\frac{1}{5}$, $\frac{1}{10}$, $\frac{1}{25}$, and $\frac{1}{50}$		1594 (Liquefying colonies more numerous.)
21.7.1893	1 I		4	$\frac{1}{5}$ and $\frac{1}{10}$	58 (Few liquefying colonies.)	
		1 R	3	$\frac{1}{6}$ and $\frac{1}{12}$		840 (Liquefying colonies more numerous.)

In this unsterilised uninfected Loch Katrine water it will be seen then that distinct, but only very restricted, multiplication took place, which was, as usual, followed by subsequent decline.

The examinations by phenol-broth culture of the uninfected unsterilised Loch Katrine water will be best considered along with the similar examinations made of the infected unsterilised waters (see p. 472, *et seq.*).

2. *Bacteriological Examination of the Infected Unsterilised Loch Katrine Water.*

The Loch Katrine waters, infected with typhoid and the *B. coli communis* respectively, were periodically examined, both by gelatine-plate and phenol-broth culture, with the following results :—

Typhoid-infected Unsterilised Loch Katrine Water. (First Series.) Infected 4.7.1893.

Dates on which plate cultivations were made.	Particular flask employed.		Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies obtained from 1 c.c. of water.	
	Incubator.	Refrigerator.			Incubator flask.	Refrigerator flask.
4.7.1893	Before subdivision.		4	c.c. $\frac{1}{12}$ and $\frac{1}{60}$	690 (Few liquefying colonies.)	
10.7.1893	1 I		3	$\frac{1}{25}$ and $\frac{1}{50}$	1425 (Numerous liquefying colonies; no colonies unmistakably like typhoid.)	
		1 R	3	$\frac{1}{25}$ and $\frac{1}{50}$		8125 (Very numerous liquefying colonies; no colonies unmistakably like typhoid.)
17.7.1893	1 I		2	$\frac{1}{30}$ and $\frac{1}{60}$	2250 (Very numerous liquefying colonies; no surface colonies with marked resemblance to typhoid.)	
		1 R	2—3	$\frac{1}{25}$ and $\frac{1}{50}$		5000 (Very numerous liquefying colonies; no surface colonies with marked resemblance to typhoid.)
21.7.1893	1 I		2	$\frac{2}{11}$ and $\frac{1}{11}$	4400 (Very numerous liquefying colonies.)	
		1 R	2—3	$\frac{1}{5}$ and $\frac{1}{10}$		4105 (Very numerous liquefying colonies.)

In this case there was a very considerable increase in the total number of bacteria present, due to the extensive multiplication of the water bacteria, as shown by the great increase in the number of liquefying colonies. As this multiplication is much more marked than in the uninfected water, it must obviously have been promoted by the small quantity of organic matter unavoidably introduced along with the typhoid bacilli.

Similarly the plate cultivations of the unsterilised Loch Katrine water infected with the *B. coli communis* yielded the following results :—

Coli-infected Unsterilised Loch Katrine Water.

Dates on which plate cultivations were made.	Particular flask employed.		Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies obtained from 1 c.c. of water.	
	Incubator.	Refrigerator.			Incubator flask.	Refrigerator flask.
4.7.1893	Before subdivision.		3—4	c.c. $\frac{1}{5}$, $\frac{1}{10}$, $\frac{1}{30}$, $\frac{1}{60}$	3350 (Only few liquefying colonies; numerous surface colonies like those of typhoid or coli.)	
11.7.1893	1 I		3	$\frac{1}{6}$, $\frac{1}{12}$, $\frac{1}{25}$, $\frac{1}{50}$	1900 (Few liquefying colonies; only few surface colonies like typhoid or coli.)	
		1 R	3	$\frac{2}{9}$, $\frac{1}{9}$, $\frac{1}{25}$, $\frac{1}{50}$		5400 (Numerous liquefying colonies, no surface colonies like typhoid or coli.)
18.7.1893	1 I		4	$\frac{1}{5}$ and $\frac{1}{10}$	63 (Very few colonies at all; one surface colony just like typhoid or coli.)	
		1 R	3	$\frac{1}{5}$ and $\frac{1}{10}$		3825 (Few liquefying colonies, some surface colonies like typhoid or coli.)
21.7.1893	1 I		3	$\frac{2}{9}$ and $\frac{1}{9}$	72 (Few liquefying colonies; no surface colonies like typhoid or coli.)	
		1 R	3	$\frac{1}{6}$ and $\frac{1}{12}$		4029 (A number of liquefying colonies, and some surface colonies like typhoid or coli.)

In the water, therefore, kept at the summer temperature there was a continuous decline in the total number of bacteria, nor was there, apparently, any multiplication of the water forms; whilst in the water kept at a winter temperature, not only was there a slight numerical increase, but also, obviously, a considerable multiplication of the water-bacteria as evidenced by the increase in the number of liquefying colonies.

In the following tables are recorded the results of the examinations by phenol-broth culture of the several unsterilised Loch Katrine waters, both infected and uninfected :—

Examination of Unsterilised Loch Katrine Waters (First Series) by Phenol Broth-culture, 8.7.1893.

Number of broth tube.	Water used for cultivation with phenol broth.	Quantity of water taken in c.c.	Quantity of phenol solution added to 10 c.c. broth.	Remarks.
		Uninfected Unsterilised Loch Katrine.		
(120)	Flask 1 I ..	1·0	3 drops	Turbid in 48 hours.
(129)	,, ..	,,	5 ,,	Did not go turbid.
(121)	Flask 1 R ..	,,	3 ,,	Turbid in 48 hours.
(130)	,, ..	,,	5 ,,	Turbid in 48 hours. Plates poured 10.7.1893.
		Typhoid-infected Unsterilised Loch Katrine.		
(122)	Flask 1 I ..	1·0	3 drops	Turbid in 48 hours.
(131)	,, ..	,,	5 ,,	Turbid in 48 hours. Plates poured 10.7.1893.
(123)	Flask 1 R ..	,,	3 ,,	Turbid in 48 hours.
(132)	,, ..	,,	5 ,,	Turbid in 48 hours. Plates poured 10.7.1893.
		Coli-infected Unsterilised Loch Katrine.		
(126)	Flask 1 I ..	1·0	3 drops	Turbid in 24 hours.
(135)	,, ..	,,	5 ,,	Turbid in 24 hours. Plates poured 9.7.1893.
(127)	Flask 1 R ..	,,	3 ,,	Turbid in 24 hours.
(136)	,, ..	,,	5 ,,	Turbid in 24 hours. Plates poured 9.7.1893.

From the above table it will be seen that all the waters rendered the phenol broth-tubes turbid, those infected with the *B. coli com-*

munis in twenty-four hours, the uninfected and typhoid-infected waters in forty-eight hours.

Of the plate cultivations prepared from these turbid broth-tubes, it need only be stated that the plates from the tubes which had gone turbid with typhoid-infected water, yielded characteristic typhoid colonies which satisfied all the several confirmatory tests; similarly the plates prepared from those broth-tubes which had been rendered turbid by coli-infected water, yielded the characteristic colonies of the *B. coli communis*, and also satisfied the various confirmatory tests. On the other hand, those phenol broth-tubes which had become turbid through the uninfected water, yielded colonies on the plates which were small in the depth, and formed small pin-heads on the surface of the gelatine, but did not give rise to the characteristic expansions, whilst on transferring these to potatoes, strong, highly-raised, greyish growths, much more conspicuous than those of typhoid, were obtained.

Thus on July 8, 1893, *four days after infection, both the typhoid bacillus and the* B. coli communis *were proved to be still alive in the unsterilised Loch Katrine water.*

The second examination by phenol broth-culture was made on July 15, 1893, or eleven days after infection, with the following results:—

Examination of Unsterilised Loch Katrine Waters by Phenol Broth-culture, 15.7.1893.

Number of broth tube.	Water used for cultivation with phenol broth.	Quantity of water taken in c.c.	Quantity of phenol solution added to 10 c.c. broth.	Remarks.
		Uninfected Unsterilised Loch Katrine.		
(140)	Flask 1 I ..	1·0	3 drops	Turbid in 48 hours. Plates poured 18.7.1893.
(146)	" ..	"	5 "	Did not go turbid.
(141)	Flask 1 R ..	"	3 "	Turbid in 48 hours. Plates poured 18.7.1893.
(147)	" ..	"	5 "	Did not go turbid.
		Typhoid-infected Unsterilised Loch Katrine.		
(142)	Flask 1 I ..	1·0	3 drops	Turbid in 72 hours. Plates poured 18.7.1893.
(148)	" ..	"	5 "	Did not go turbid.
(143)	Flask 1 R ..	"	3 "	Turbid in 24 hours. Plates poured 18.7.1893.
(149)	" ..	"	5 "	Did not go turbid.
		Coli-infected Unsterilised Loch Katrine.		
(144)	Flask 1 I ..	1·0	3 drops	Turbid in 24 hours.
(150)	" ..	"	5 "	Turbid in 48 hours. Plates poured 17.7.1893.
(145)	Flask 1 R ..	"	3 "	Turbid in 24 hours.
(151)	" ..	"	5 "	Turbid in 48 hours.

Of the plate cultivations made from the above turbid broth-tubes, it will be sufficient to say:—

1. That from the uninfected water only liquefying colonies were obtained, probably *B. liquidus* (Percy Frankland); as already mentioned, this organism is very frequently obtained in phenol broth-cultivations in which only 3 drops of phenol solution has been added.

2. The plates from the phenol broth-tube which had only been rendered turbid in seventy-two hours by the incubator flask of the typhoid-infected water, yielded also only liquefying colonies, and nothing like typhoid colonies was discoverable on the plates. The corresponding broth-tube from the refrigerator flask, on the other hand, which had become turbid in twenty-four hours, yielded numerous small and expansion colonies which were undoubtedly due to typhoid.

3. The broth-tube, which had been rendered turbid in twenty-four hours with coli-infected water, yielded plates containing numerous depth and surface-expansion colonies, which were undoubtedly those of the *B. coli communis*.

Thus from these examinations it was apparent that on July 15, 1893, *or eleven days after infection, the* Bacillus coli communis *was still alive in the unsterilised Loch Katrine water, as was also the typhoid bacillus in similar water which had been kept at the winter temperature of* 6—8° *C., whilst in the water kept at a summer temperature of* 19° *C. the typhoid bacillus was no longer discoverable.*

Another examination was made of these uninfected and infected unsterilised Loch Katrine waters on July 21, 1893, with the following results:—

Examination of Unsterilised Loch Katrine Waters by Phenol Broth-culture, 21.7.1893.

Number of broth tube.	Water used for cultivation with phenol broth.	Quantity of water taken in c.c.	Quantity of phenol solution added to 10 c.c. broth.	Remarks.
		Uninfected Unsterilised Loch Katrine.		
(175)	Flask 1 I ..	1·0	3 drops	Only turbid after 8 days.
(176)	„ 1 R..	„	„	Only turbid after 4 days.
		Typhoid-infected Unsterilised Loch Katrine.		
(177)	Flask 1 I ..	1·0	3 drops	Turbid in 4 days. Plates poured 25.7.1893.
(178)	„ 1 R..	„	„	Turbid in 48 hours. Plates poured 23.7.1893.
		Coli-infected Unsterilised Loch Katrine.		
(179)	Flask 1 I ..	1·0	3 drops	Turbid in 48 hours. Plates poured 23.7.1893.
(180)	„ 1 R..	„	„	Turbid in 48 hours. Plates poured 23.7.1893.

The plate cultivations made from the above turbid broth-tubes yielded the same results as those on July 15, 1893; thus no typhoid colonies were obtained on the plates from broth-tube No. 177, whilst they were easily discoverable and confirmed on the plates from broth-tube No. 178; again the colonies of the *B. coli communis* were

readily detected and confirmed on the plates from both broth-tubes Nos. 179 and 180.

From these examinations it was evident, therefore, that the B. coli communis *was still alive in the unsterilised Loch Katrine waters (kept both at winter and summer temperature) on July* 21, 1893, *or seventeen days after infection; the typhoid bacillus was also still alive in the similar water kept at the winter temperature* (6—8° *C.*), *whilst it was again, as on the previous occasion* (July 15, 1893), *proved to be extinct in the same water kept at the summer temperature of* 19° *C.*

3. *Bacteriological Examination of the Infected Sterilised Loch Katrine Waters.*

With the preceding results must now be compared the behaviour of the typhoid bacillus and the *B. coli communis* in the Loch Katrine water which had been previously sterilised by steam and by filtration through porous porcelain respectively.

These infected sterile waters were, as before, intended to show whether these bacilli are capable of multiplication or not in water of this character when the disturbing influence of the simultaneous presence of other micro-organisms is removed.

Typhoid-infected Steam Sterilised Loch Katrine Water. (First Series.)

Dates on which plate cultivations were made.	Particular flask employed.		Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies obtained from 1 c.c. of water.	
	Incubator.	Refrigerator.			Incubator flask.	Refrigerator flask.
4.7.1893	Before subdivision.		6	c.c. $\frac{1}{4}$ and $\frac{1}{12}$	720	
10.7.1893	1 I		7	$\frac{1}{3}$ and $\frac{1}{9}$	77	
		1 R	7	$\frac{1}{4}$ and $\frac{1}{12}$		228
17.7.1893	1 I		7—8	1·0 and $\frac{3}{10}$	15	
		1 R	8	$\frac{5}{6}$ and $\frac{1}{4}$		29
21.7.1893	1 I		5	1·0 and 0·5	0	
		1 R	5	$\frac{5}{6}$ and $\frac{5}{12}$		1
25.7.1893	1 I		6	$\frac{5}{6}$ and $\frac{5}{12}$	0	
		1 R	6	1·0 and 0·5		2

Thus in this steam-sterilised Loch Katrine water the typhoid bacilli underwent rapid degeneration, the rate of their decline being more rapid at the summer than at the winter temperature; for at the higher temperature they were no longer demonstrable seventeen days after infection, whilst at the lower temperature they were still just discoverable even after twenty-one days.

Essentially similar was the behaviour of the typhoid bacilli in the Loch Katrine water which had been sterilised by filtration through porous porcelain, thus:—

Typhoid-infected Porcelain-filtered Loch Katrine Water. (First Series.)

Dates on which plate cultivations were made.	Particular flask employed.		Number of days plates were incubated.	Volume of water employed for cultivation.	Number of colonies obtained from 1 c.c. of water.	
	Incubator.	Refrigerator.			Incubator flask.	Refrigerator flask.
4.7.1893	Before subdivision.		6	c.c. $\frac{3}{11}$ and $\frac{1}{11}$	615	
10.7.1893	1 I		7	$\frac{3}{13}$ and $\frac{1}{13}$	130	
		1 R	7	$\frac{3}{10}$ and $\frac{1}{10}$		345
17.7.1893	1 I		8	$\frac{5}{6}$ and $\frac{1}{4}$	50	
		1 R	8	$\frac{5}{6}$ and $\frac{1}{4}$		100
21.7.1893	1 I		5	$\frac{10}{11}$ and $\frac{5}{11}$	1 (?) Plate contaminated.	
		1 R	5	$\frac{5}{6}$ and $\frac{5}{12}$		2 (?) Plate much contaminated.
25.7.1893	1 I		6	1·0 and 0·5	0 Not contaminated.	
		1 R	6	1·0 and 0·5		20 Not contaminated.

Thus again in the Loch Katrine water, sterilised by filtration through porous porcelain, the typhoid bacilli underwent rapid degeneration, more especially in the water which was preserved at the summer temperature, in which they were no longer found by plate cultivation twenty-one days after infection, whilst in the same water kept at the winter temperature they were still easily recognisable, although in greatly diminished numbers, on that day.

It is particularly noteworthy that the behaviour of the typhoid bacilli was practically identical in this Loch Katrine water, irrespectively of whether it was employed in the unsterilised or in the sterilised condition, and irrespectively of whether the sterilisation was effected by steam or by filtration through porous porcelain.

In all cases, moreover, the effect of temperature on the typhoid bacillus was very marked, the longevity being much greater in the Loch Katrine water, unsterilised or sterilised, kept at the winter than in that kept at the summer temperature.

The behaviour of the *B. coli communis* in these sterilised L. Katrine waters is recorded in the following tables :—

Coli-infected Steam-sterilised Loch Katrine Water.

Dates on which plate cultivations were made.	Particular flask employed.		Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies obtained from 1 c.c. of water.	
	Incubator.	Refrigerator.			Incubator flask.	Refrigerator flask.
4.7.1893	Before subdivision.		6	c.c. $\frac{1}{4}$ and $\frac{1}{12}$	2,180	
11.7.1893	1 I		6	$\frac{3}{11}$ and $\frac{1}{11}$	0	
		1 R	6	$\frac{1}{4}$ and $\frac{1}{12}$		162
18.7.1893	1 I		7	2·0 and 1·0	1	
		1 R	7	2·0 and 1·0		49
21.7.1893	1 I		5	2·0 and 1·0	0	
		1 R	5	$\frac{3}{5}$ and $\frac{1}{5}$		0
25.7.1893	1 I		6	2·0 and 1·0	0	
		1 R	6	$\frac{1}{2}$ and $\frac{1}{6}$		0
26.7.1893	1 I		5	4·0 and 3·0	0	
		1 R	5	4·0 and 2·0		0

Thus in the steam-sterilised L. Katrine water, the B. coli communis *disappeared in a surprisingly short time, being no longer demonstrable on the* 17*th day after infection. In this case also the decline was more rapid in the water kept at a summer than in that at a winter temperature.*

Very similar again was the behaviour of the *B. coli communis* in the L. Katrine water previously sterilised by filtration through porous porcelain, thus :—

Coli-infected Porcelain-filtered Loch Katrine Water.

Dates on which plate cultivations were made.	Particular flask employed.		Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies obtained from 1 c.c. of water.	
	Incubator.	Refrigerator.			Incubator flask.	Refrigerator flask.
4.7.1893	Before subdivision.		6	c.c. $\frac{3}{13}$ and $\frac{1}{13}$	1,900	
11.7.1893	1 I	1 R	6 6	$\frac{3}{10}$ and $\frac{1}{10}$ $\frac{3}{10}$ and $\frac{1}{10}$	0	15
18.7.1893	1 I	1 R	7 7	1·0 and 0·5 2·0 and 1·0	0	3
21.7.1893	1 I	1 R	5 5	2·0 and 1·0 2·0 and 1·0	0	1
25.7.1893	1 I	1 R	6 6	2·0 and 1·0 2·0 and 1·0	4	1
26.7.1893	1 I		5	4·0 and 3·0	0	

Thus again in the case of this porcelain-filtered L. Katrine water there was the same rapid disappearance of the introduced B. coli communis.

It is particularly remarkable that the B. coli communis *has disappeared more rapidly in both these sterile L. Katrine waters than in the unsterilised.*

Similar evidence of the disappearance of the typhoid and coli bacilli in these sterile L. Katrine waters is afforded by the results of the several examinations by phenol broth-culture, thus :—

Examinations of Typhoid-infected Sterilised Loch Katrine Waters by Phenol Broth-culture.

Date and number of broth tube.	Water used for cultivation with phenol broth.	Volume of water taken in c.c.	Quantity of phenol solution added to 10 c.c. broth.	Remarks.
8.7.1893	*Typhoid-infected Steam-sterilised Loch Katrine.*			
(137)	Flask 1 R ..	1 drop	3 drops	Turbid in 24 hours.
17.7.1893				
(162)	Flask 1 I ..	0·5	3 drops	Turbid in 24 hours.
(163)	,, ..	1·0	,,	,, ,,
20.7.1893				
(172)	Flask 1 I ..	0·5	3 drops	Did not go turbid.
(173)	,, ..	1·0	,,	,, ,,
25.7.1893				
(207)	Flask 1 I ..	1·0	3 drops	Turbid in 4 days.
(208)	,, 1 R ..	,,	,,	Did not go turbid.
	Typhoid-infected Porcelain-filtered Loch Katrine.			
(209)	Flask 1 I ..	1·0	3 drops	Did not go turbid.
(210)	,, 1 R ..	,,	,,	Turbid in 48 hours.
26.7.1893	*Typhoid-infected Steam-sterilised Loch Katrine.*			
(220)	Flask 1 I ..	1·0	3 drops	Did not go turbid.
(221)	,, 1 R ..	,,	,,	,, ,,

Examinations of Coli-infected Sterilised Loch Katrine Waters by Phenol Broth-culture.

Date and number of broth tube.	Water used for cultivation with phenol broth.	Volume of water taken in c.c.	Quantity of phenol solution added to 10 c.c. broth.	Remarks.
21.7.1893.	*Coli-infected Steam-sterilised Loch Katrine.*			
(195)	Flask 1 I ..	2·0	3 drops	Did not become turbid.
(196)	" ..	1·0	"	" "
	Coli-infected Porcelain-filtered Loch Katrine.			
(197)	Flask 1 I ..	2·0	3 drops	Did not become turbid.
(198)	" ..	1·0	"	" "
(199)	Flask 1 R ..	2·0	"	Turbid in 48 hours.
(200)	" ..	1·0	"	Did not become turbid.
25.7.1893.	*Coli-infected Steam-sterilised Loch Katrine.*			
(203)	Flask 1 I ..	1·0	3 drops	Did not become turbid.
(204)	" 1 R ..	1·0	"	" "
	Coli-infected Porcelain-filtered Loch Katrine.			
(205)	Flask 1 I ..	1·0	3 drops	Turbid in 48 hours.
(206)	" 1 R ..	1·0	"	" "
26.7.1893. (222)	Flask 1 I ..	1·0	3 drops	Did not become turbid.

These examinations by phenol broth-culture substantiate the results obtained by gelatine plates, and show that only a very small number of the bacilli were still living in the waters on the later dates. Thus, in the case of broth-tubes Nos. 199 and 200, it is evident that in No. 199, in which 2 c.c. of water were employed, at least one living *B. coli communis* was introduced, for the broth-tube became turbid; whilst in No. 200, in which only 1 c.c. of the same water was employed, no living bacillus can have been introduced, as the broth-tube did not become turbid. On referring to the table of gelatine plate examinations (p. 483) it will be seen that on the same day (21.7.1893) in the same water there was found only one *B. coli communis* colony per 1 c.c., so that it might easily happen that any particular 1 c.c. of the water might not contain any bacillus, as was apparently the case in the 1 c.c. of this water added to the phenol broth-tube No. 200.

It is in this way that particular interest attaches to a comparison of the results obtained by gelatine plate and phenol broth-culture in the case of these infected sterile waters, as the two methods can be made to control each other, whilst in the case of the infected unsterilised waters the method of phenol broth-culture has to be exclusively relied on for the detection of the typhoid and coli bacilli.

Behaviour of the Typhoid Bacillus in the Loch Katrine Water. (Second Series of Experiments.)

In the first series of experiments with the L. Katrine water recorded above, the number of typhoid bacilli initially introduced was so small that it would obviously not be possible to directly compare the results with those previously obtained with Thames water in which a much larger number of typhoid bacilli were initially introduced, as I have found in previous investigations of the same kind that one of the factors determining the longevity of pathogenic bacteria placed in water, or for the matter of that placed in any unfavourable surroundings, is the absolute number in which they are present. In other words, amongst, for instance, 1,000 bacteria taken from a given source there may be *some individuals* which will resist a particular adverse influence, whilst amongst 10 bacteria taken from the same source there may be *none* capable of resisting the adverse influence in question.

When, therefore, I found that such a small number of typhoid bacilli had been introduced into the L. Katrine water in the first series of experiments, I immediately started a second series of experiments with the same water, but introducing a much larger number of typhoid bacilli.

In this second series of Loch Katrine experiments, which were begun on 7.7.1893, or three days after the first, only unsterilised Loch Katrine water was infected with typhoid, thus :—

Infection of L. Katrine Water in Second Series of Experiments.— 25 needle-loops were taken from the surface of an agar-culture of the typhoid bacillus, 11 days old, and introduced into 20 c.c. of steam-sterilised tap-water, which was then violently shaken for 15 minutes; 10 c.c. of this water-attenuation were then added to 1500 c.c. of unsterilised L. Katrine water. After thorough mixture this was divided up amongst a number of sterilised flasks plugged with sterile cotton-wool, which were placed in the incubator (19° C.) and refrigerator (6—8° C.) respectively. Control flasks containing the same unsterilised L. Katrine water, but uninfected, were placed under precisely similar conditions.

The results of bacteriological examination of the uninfected control L. Katrine water are given in the following table :—

Unsterilised Uninfected Loch Katrine Water. (Second Series.)

Dates on which plate cultivations were made.	Particular flask employed.		Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies obtained from 1 c.c. of water.	
	Incubator.	Refrigerator.			Incubator flask.	Refrigerator flask.
7.7.1893	Before subdivision.		3	c.c. $\frac{2}{11}$ and $\frac{1}{11}$	410	
11.7.1893	1 I		3	$\frac{1}{6}$, $\frac{1}{95}$, $\frac{1}{30}$, $\frac{1}{60}$	4550	
		1 R	3	$\frac{1}{5}$, $\frac{1}{10}$, $\frac{1}{25}$, $\frac{1}{50}$		412
18.7.1893	1 I		6	$\frac{2}{9}$ and $\frac{1}{9}$	39	
		1 R	3	$\frac{1}{5}$ and $\frac{1}{10}$		335
21.7.1893	1 I		3	$\frac{2}{13}$ and $\frac{1}{13}$	114	
		1 R	3—4	$\frac{1}{6}$ and $\frac{1}{12}$		657
					N.B.—Only a small number of liquefying colonies on any of the above plates.	

Typhoid-infected Unsterilised Loch Katrine Water. (Second Series.)

Dates on which plate cultivations were made.	Particular flask employed.		Number of days plates were incubated.	Volume of water employed for plate cultivations.	Number of colonies obtained from 1 c.c. of water.	
	Incubator.	Refrigerator.			Incubator flask.	Refrigerator flask.
7.7.1893	Before subdivision.		3	c.c. $\frac{1}{30}$ and $\frac{1}{60}$	441,000	
11.7.1893	1 I		3	$\frac{1}{25}$ and $\frac{1}{50}$	298,000 (Plates badly liquefied, so that numbers somewhat uncertain, and doubtless under-estimated.)	
		1 R	3	$\frac{1}{25}$ and $\frac{1}{50}$		250,000 (Plates also badly liquefied. This liquefaction indicates that the water-bacteria must have undergone great multiplication.)
18.7.1893	1 I		4	$\frac{1}{50}$	8250 (A few surface colonies, doubtless typhoid.)	
		1 R	3	$\frac{1}{25}$ and $\frac{1}{50}$		253,000 (Very numerous surface colonies, doubtless typhoid.)
					In the plates from both waters a number of liquefying colonies present.	
21.7.1893	1 I		2	$\frac{1}{30}$ and $\frac{1}{60}$	3800 (Several expansion colonies, doubtless typhoid.)	
		1 R	3	$\frac{1}{25}$ and $\frac{1}{50}$		3000 (Several expansion colonies, doubtless typhoid.)
					The plates from both waters contained a number of liquefying colonies.	

Thus in these uninfected L. Katrine waters only comparatively slight multiplication took place, and at no time were there more than a few colonies causing liquefaction of the gelatine.

With these must now be compared the L. Katrine water infected with typhoid, the results obtained with which are recorded in the table (p. 488).

These examinations show that although the water-bacteria present in the unsterilised L. Katrine water must have undergone considerable multiplication, as shown by the great increase in the liquefying colonies, yet this multiplication did not by any means keep pace with the decrease in the number of typhoid bacilli; for the total number of colonies on the successive plates underwent continuous decline.

The actual proof of the persistence of the typhoid bacillus in these waters had of course to be furnished by the method of phenol broth-culture. Thus

Examination of Unsterilised Loch Katrine Waters (Second Series) by Phenol Broth-culture.

Number of broth tube.	Water used for cultivation with phenol broth.	Volume of water taken in c.c.	Quantity of phenol solution added to 10 c.c. broth.	Remarks.	
				Typhoid-infected Unsterilised Loch Katrine (Second Series).	
8.7.1893					
(124)	Flask 1 I ..	1 0	3 drops	Turbid in 48 hours.	On plate cultivation the tubes Nos. 133 and 134 yielded typical typhoid colonies, which were further confirmed by usual tests. *Typhoid present.*
(133)	,, ..	,,	5 ,,	,, ,,	
(125)	Flask 1 R ..	,,	3 ,,	,, ,,	
(134)	,, ..	,,	5 ,,	,, ,,	
15.7.1893					
(154)	Flask 1 I ..	,,	3 ,,	Turbid in 72 hours.	On plate cultivation the tubes Nos. 155, 158 and 159, yielded typical typhoid colonies, which were further confirmed by usual tests. *Typhoid present.*
(158)	,, ..	,,	5 ,,	,, ,,	
(155)	Flask 1 R ..	,,	3 ,,	Turbid in 24 hours.	
(159)	,, ..	,,	5 ,,	,, 72 ,,	
				Uninfected Unsterilised Loch Katrine (Second Series).	
(152)	Flask 1 I ..	1·0	3 drops	Turbid in 48 hours.	Plate cultivations of the tubes Nos. 152 and 157 yielded somewhat expanding milk-drop colonies, and which were further differentiated from typhoid by giving bubbles in gelatine, and thick growths on potatoes, but no indol or milk reactions.
(156)	,, ..	,,	5 ,,	Did not become turbid.	
(153)	Flask 1 R ..	,,	3 ,,	Turbid in 48 hours.	
(157)	,, ..	,,	5 ,,	,, ,,	

Typhoid-infected Unsterilised Loch Katrine (Second Series).

21.7.1893					
(189)	Flask 1 I ..	1·0	3 drops	Turbid in 24 hours.	Plate cultivations of the tube No. 193 gave colonies which were recognised and confirmed as typhoid, besides other colonies resembling the *B. coli communis*, and answering to the same tests (gas bubbles, indol, milk, and potatoes). The plate cultivations of tube No. 194, on the other hand, gave only typhoid colonies which were duly confirmed. *Typhoid present.*
(193)	,, ..	,,	5 ,,	,, 48 ,,	
(190)	Flask 1 R ..	,,	3 ,,	,, ,, ,,	
(194)	,, ..	,,	5 ,,	,, ,, ,,	

Uninfected Unsterilised Loch Katrine (Second Series).

(187)	Flask 1 I ..	1·0	3 drops	Turbid in 72 hours.
(191)	,, ..	,,	5 ,,	Only turbid in 8 days.
(188)	Flask 1 R ..	,,	3 ,,	Turbid in 72 hours.
(192)	,, ..	,,	5 ,,	Only turbid in 8 days.

Thus when the typhoid bacilli were introduced into the L. Katrine water in large numbers, they were still easily discoverable by phenol broth-culture on the fourteenth day, although from the examinations by plate-cultivation (see p. 488) it is obvious that their numbers had undergone enormous diminution. They doubtless persisted even longer than this, but the experiments had to be interrupted. Thus when introduced in large numbers their persistence is greater than when only small numbers are employed, for in the previous experiments they were no more demonstrable in the unsterilised water which had been kept at a summer temperature (19° *C.*) *for* 11 *days* (*see p.* 476).

Comparative Behaviour of the Typhoid Bacillus in Thames, Loch Katrine, and Deep Well Water.

The previous experiments had clearly shown that the typhoid bacillus, although unable to multiply in either ordinary Thames or L. Katrine water, even when these waters are deprived of other competing or inimical bacteria, is yet able to remain alive for considerable periods of time in these waters, not only when they are previously sterilised, but even, although for a distinctly shorter period, in their unsterilised condition and in the presence of an abundant bacterial population.

Inasmuch as the access of typhoid bacilli to potable water of all kinds is one of the most ever-present dangers to the public health, it becomes a matter of pressing hygienic importance to determine whether the particular kind of water into which they may gain access affects the chance of their reaching the water-consumer in a living state. The population of the United Kingdom is chiefly supplied with one or other of three different kinds of water, of which the Thames, L. Katrine, and deep-well water of the Kent Company may be taken as types, and it is with these three types of water that I have, therefore, instituted the comparison in question.

From the experiments which I have detailed above, it is obvious that the longevity of the typhoid bacillus in any particular water is subject to very considerable variations according to the initial vitality of the typhoid bacillus employed, and according as a relatively large or small number of the bacilli is introduced into the water. In order, therefore, to institute a comparison between several different waters as to their relative capacity of maintaining the typhoid bacilli in a living state, it is absolutely essential that the typhoid bacilli placed in the several waters should be taken from one and the same cultivation, and that they should be introduced in each case in as far as possible the same numbers.

These were the conditions which were secured in the series of comparative experiments made with these three different types of water, and which are now to be described.

Simultaneous Infection with Typhoid of Thames, Loch Katrine, and Deep Well Water.

Each of these waters was, in this comparative series, simultaneously experimented with in the natural unsterilised state, also after sterilisation by steam, as well as after sterilisation by filtration through a porous cylinder composed of baked infusorial earth. These nine different kinds of water were all infected at one time with the same quantity of typhoid bacilli taken from one and the same cultivation.

For this purpose an agar-cultivation of sixteen days' age, and grown at 18—20° C., was employed. Forty needle loops were carefully taken from the surface of this cultivation and thoroughly mixed by prolonged agitation with 50 c.c. of sterilised tap-water. Of the water-attenuation thus prepared 4 c.c. were added to 1000 c.c. of each of the nine different kinds of water. In this manner was, therefore, secured the equal infection both qualitatively and quantitatively of each of the nine experimental waters. Each of these infected waters was subdivided amongst several sterile flasks. The mouths of these flasks instead of being plugged with cotton-wool, were in this series of experiments simply covered with sterile beakers, an arrangement which is in many respects preferable for purposes of this kind. All these flasks, together with similar flasks containing each of the three unsterilised waters not infected, were placed in a dark cupboard in which there prevailed an almost uniform temperature of 9—12° C.

In this series of experiments, besides determining the relative longevity of the typhoid bacilli in the several different types of potable water, I have also endeavoured to ascertain the effect on the bacteria of keeping the waters at rest and in motion respectively. To this end, in the case of each water, one flask was kept at rest and only shaken up when a sample was to be taken from it, whilst the other flask was daily submitted to violent agitation over a period of five minutes, this agitation being repeated two or three times on the same day. The convention will be adopted in the following pages of referring to the flasks kept at rest by the letter A, whilst those which were subjected to daily agitation are distinguished by the letter B.

The various waters were periodically examined both by plate-cultivation and phenol-broth culture on the same lines as described for the previous series of experiments.

The uninfected waters yielded the following results on chemical analysis:—

Results of Analysis expressed in Parts per 100,000.

	Thames water collected 5.10.1893.	Deep well water (Kent waterworks), collected 6.10.1893.	Loch Katrine water, collected 13.10.1893.
Total solid matter	26·20	44·40	2·40
Organic carbon } by combustion	0·172	0·058	0·189
Organic nitrogen } by combustion	0·036	—	0·024
Organic nitrogen (Kjeldahl process)	0·46	0·013	0·016
Ammonia (free)	0·005	0	0
Ammonia (albuminoid)	0·013	0·001	0·001
Oxygen consumed by organic matter	0·083	0·013	0·147
Nitrogen as nitrates and nitrites	0·175	0·467	0·007
Total combined nitrogen	0·215	0·480	0·031
Chlorine	1·75	2·50	0·65
Temporary hardness	4·2	21·1	0
Permanent "	13·7	8·6	1·0
Total "	17·9	29·7	1·0
	Turbid	Clear	Very slightly turbid

Comparison of Thames, Loch Katrine, and Deep Well Waters.

Uninfected Unsterilised Thames Water.

Dates on which plate cultivations were made.	Particular flask employed.		Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies obtained from 1 c.c. of water.	
	Kept at rest.	Daily agitated.			Flask kept at rest.	Flask daily agitated.
19.10.1893	Before subdivision.		8	c.c. $\frac{1}{50}$ and $\frac{1}{100}$	1,350 Very few liquefying colonies.	
1.11.1893	A		3—6	$\frac{1}{50}$ and $\frac{1}{100}$	4,600	
		B	4	$\frac{1}{60}$ and $\frac{1}{120}$		10,000
7.11.1893	A		6	$\frac{1}{50}$ and $\frac{1}{100}$	11,900	
		B	6	$\frac{1}{30}$ and $\frac{1}{100}$		6,900

Typhoid-infected Unsterilised Thames Water.

19.10.1893	Before subdivision.		5	$\frac{1}{50}$ and $\frac{1}{100}$	17,900 Hardly any liquefying colonies, nearly all typhoid.	
1.11.1893	A		3	$\frac{1}{50}$ and $\frac{1}{100}$	3,800	
		B	4—6	$\frac{1}{60}$ and $\frac{1}{120}$		5,800
7.11.1893	A		3	$\frac{1}{50}$ and $\frac{1}{100}$	4,000 Many liquefying colonies.	
		B	4	$\frac{1}{50}$ and $\frac{1}{100}$		4,600

The above tables show that the bacteria in the uninfected Thames water underwent very considerable multiplication, and in the typhoid-infected water, although the total number of bacteria declined, it is certain that the water-bacteria underwent considerable multiplication, as there was a great increase in the number of liquefying colonies; the diminution in the total number of bacteria was due to the disappearance of the typhoid bacilli which were initially present to the extent of about 16,000 per c.c. *It will be shown (p. 508) that typhoid bacilli were no longer demonstrable in this water by phenol broth culture after* 28.10.1893, *or nine days after their introduction into the Thames water.*

Comparison of Thames, Loch Katrine, and Deep Well Waters.

Uninfected Unsterilised Loch Katrine Water.

Dates on which plate cultivations were made.	Particular flask employed.		Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies obtained from 1 c.c. of water.	
	Kept at rest.	Daily agitated.			Flask kept at rest.	Flask daily agitated.
19.10.1893	Before subdivision.		5—6	c.c. $\frac{1}{30}$ and $\frac{1}{60}$	1,625	
1.11.1893	A		6	$\frac{1}{30}$ and $\frac{1}{60}$	1,025	
		B	4	$\frac{1}{30}$ and $\frac{1}{60}$		1,400
7.11.1893	A		6	$\frac{1}{30}$ and $\frac{1}{60}$	750	
		B	4	$\frac{1}{25}$ and $\frac{1}{50}$		360
21.11.1893	A		4	$\frac{1}{25}$ and $\frac{1}{50}$	450	Not further examined.
27.11.1893	A		4	$\frac{1}{25}$ and $\frac{1}{50}$	1,200	Not further examined.

Typhoid-infected Unsterilised Loch Katrine Water.

Dates on which plate cultivations were made.	Kept at rest.	Daily agitated.	Number of days plates were incubated.	Volume of water employed for plate cultivation.	Flask kept at rest.	Flask daily agitated.
19.10.1893	Before subdivision.		3—4	$\frac{1}{25}$ and $\frac{1}{50}$	24,000	
1.11.1893	A		3	$\frac{1}{25}$ and $\frac{1}{50}$	6,425	
		B	3	$\frac{1}{25}$ and $\frac{1}{50}$		6,600
7.11.1893	A		3	$\frac{1}{25}$ and $\frac{1}{50}$	9,000	
		B	3—4	$\frac{1}{25}$ and $\frac{1}{50}$		2,900
21.11.1893	A		3	$\frac{1}{25}$ and $\frac{1}{50}$	6,800	Not further examined.
27.11.1893	A		2	$\frac{1}{25}$ and $\frac{1}{50}$	7,800	Not further examined.

From the above tables it will be seen that the bacteria in the uninfected Loch Katrine water underwent no multiplication, but, on the contrary, slight decline. In the infected water the initial number of typhoid bacilli must have amounted to about 22,000 per c.c., and although the total number of bacteria in this infected water underwent a great decline, there can be no doubt that the water-bacteria multiplied considerably, as was evidenced by the increased number of liquefying colonies, the diminution in the total number of bacteria being doubtless due to the disappearance of the typhoid bacilli. *It will be shown on p.* 511 *that the typhoid bacilli were discovered for the last time by the method of phenol broth culture on* 7.11.1893, *or nineteen days after their first introduction.*

Comparison of Thames, Loch Katrine, and Deep Well Waters.

Uninfected Unsterilised Deep Well Water.

Dates on which plate cultivations were made.	Particular flask employed.		Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies obtained from 1 c.c. of water.	
	Kept at rest.	Daily agitated.			Flask kept at rest.	Flask daily agitated.
19.10.1893	Before subdivision.		8	c.c. $\frac{1}{50}$ and $\frac{1}{100}$	1,725	
1.11.1893	A		3	$\frac{1}{50}$ and $\frac{1}{100}$	12,000	
		B	3	$\frac{1}{60}$ and $\frac{1}{120}$		33,000
7.11.1893	A		3	$\frac{1}{50}$ and $\frac{1}{100}$	13,700	
		B	3	$\frac{1}{60}$ and $\frac{1}{120}$		20,250
21.11.1893	A		3	$\frac{1}{60}$ and $\frac{1}{120}$	3,825	Not examined further.
27.11.1893	A		2—3	$\frac{1}{50}$ and $\frac{1}{100}$	5,850	Not examined further.

Typhoid-infected Unsterilised Deep Well Water.

19.10.1893	Before subdivision.		5	$\frac{1}{50}$ and $\frac{1}{100}$	29,750	
1.11.1893	A		3	$\frac{1}{50}$ and $\frac{1}{100}$	240,000	
		B		$\frac{1}{50}$ and $\frac{1}{100}$		291,000
7.11.1893	A		3	$\frac{1}{60}$ and $\frac{1}{120}$	204,000	
		B	3	$\frac{1}{60}$ and $\frac{1}{120}$		209,000
21.11.1893	A		3	$\frac{1}{50}$ and $\frac{1}{100}$	41,000	Not examined further.
27.11.1893	A		2	$\frac{1}{60}$ and $\frac{1}{120}$	44,000	Not examined further.

From the above tables it will be seen that the water-bacteria in the deep well water underwent much more extensive multiplication than in either the Thames or Loch Katrine water. This extensive multiplication of the bacteria in such deep well water was first called attention to by me in 1886 ('Proc. Roy. Soc.'). The infected water must have contained, initially, about 28,000 typhoid bacilli per c.c., and in this infected water the increase in the total number of bacteria was even more marked than in the uninfected, the increase in the number of liquefying colonies being altogether enormous. The behaviour of the typhoid bacilli in the sterile deep well water (see p. 505) clearly shows that they cannot have participated in this bacterial multiplication observed in the infected unsterilised deep well water, but on the contrary they must have undergone great diminution, as *it will be shown on p.* 515 *that the typhoid bacilli were no longer discovered by phenol broth culture in this water after* 21.11.1893, *or thirty-three days after their first introduction.*

Comparison of Thames, Loch Katrine, and Deep Well Waters.

Typhoid-infected Steam-sterilised Thames Water.

Dates on which plate cultivations were made.	Particular flask employed. Kept at rest.	Particular flask employed. Daily agitated.	Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies obtained from 1 c.c. of water. Flask kept at rest.	Number of colonies obtained from 1 c.c. of water. Flask daily agitated.
19.10.1893	Before subdivision		5	c.c. $\frac{1}{2}$ and $\frac{1}{5}$	10,980	
30.10.1893	A		7	$\frac{8}{11}$ and $\frac{2}{11}$	4,100	
		B	7	$\frac{2}{3}$ and $\frac{1}{6}$		4,700
8.11.1893	A		6	$\frac{8}{11}$ and $\frac{2}{11}$	2,400	
		B	6	$\frac{8}{11}$ and $\frac{2}{11}$		1,850
20.11.1893	A		9	$\frac{4}{5}$ and $\frac{1}{5}$	118	
27.11.1893	A		8	$\frac{2}{3}$ and $\frac{1}{6}$	0	

Typhoid-infected Porcelain-filtered Thames Water.

Dates on which plate cultivations were made.	Particular flask employed. Kept at rest.	Particular flask employed. Daily agitated.	Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies obtained from 1 c.c. of water. Flask kept at rest.	Number of colonies obtained from 1 c.c. of water. Flask daily agitated.
19.10.1893	Before subdivision		5	$\frac{1}{2}$ and $\frac{1}{5}$	9,800	
30.10.1893	A		8	$\frac{8}{11}$ and $\frac{2}{11}$	The plates contained no colonies resembling typhoid, but a very large number of small yellow colonies very similar to colonies found in the unfiltered plates. The organism giving rise to these colonies had probably passed through the filter in small numbers, and had then undergone enormous multiplication in the filtered water.	
		B	8	$\frac{2}{3}$ and $\frac{1}{6}$		

From the above tables it will be seen that *in the steam sterilised Thames water the typhoid bacilli underwent no multiplication, but, on the contrary, steady diminution, being last discovered on* 20.11.1893, *or thirty-two days after their first introduction.*

In the Thames water, sterilised by filtration, their disappearance was far more rapid, for they were no longer discoverable eleven days after their introduction, and they had doubtless died off even before this. These results entirely confirm my previous experiences recorded on p. 464, and the confirmation is of the more importance, as the filters used in the two cases were entirely different; thus whilst that used on the former occasion was a Chamberland cylinder of porous porcelain, the one used in this latter instance was a small porous cylinder constructed of infusorial earth. These infusorial earth cylinders are much more porous than the porcelain ones, and pass the water far more rapidly.

Comparison of Thames, Loch Katrine, and Deep Well Waters.

Typhoid-infected Steam-sterilised Loch Katrine Water.

Dates on which plate cultivations were made.	Particular flask employed.		Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies obtained from 1 c.c. of water.	
	Kept at rest.	Daily agitated.			Flask kept at rest.	Flask daily agitated.
19.10.1893	Before subdivision		5	c.c. $\frac{5}{11}$ and $\frac{2}{11}$	12,000	
30.10.1893	A		7	$\frac{2}{3}$ and $\frac{1}{6}$	7,500	
		B	7	$\frac{8}{11}$ and $\frac{2}{11}$		7,200
8.11.1893	A		6	$\frac{4}{5}$ and $\frac{1}{5}$	3,800	
		B	6	$\frac{8}{11}$ and $\frac{2}{11}$		3,100
20.11.1893	A		7	$\frac{2}{3}$ and $\frac{1}{6}$	790	
27.11.1893	A		8	$\frac{8}{9}$ and $\frac{2}{9}$	220	
9.12.1893	A		9	$1\frac{1}{9}$ and $\frac{5}{9}$	250	

Typhoid-infected Porcelain-filtered Loch Katrine Water.

Dates on which plate cultivations were made.	Kept at rest.	Daily agitated.	Number of days plates were incubated.	Volume of water employed for plate cultivation.	Flask kept at rest.	Flask daily agitated.
19.10.1893	Before subdivision		5	$\frac{5}{12}$ and $\frac{1}{3}$	11,000	
30.10.1893	A		7	$\frac{8}{11}$ and $\frac{2}{11}$	9,000	
		B	7	$\frac{8}{11}$ and $\frac{2}{11}$		9,000
8.11.1893	A		6	$\frac{8}{13}$ and $\frac{2}{13}$	4,800	
		B	6	$\frac{8}{11}$ and $\frac{2}{11}$		4,100
20.11.1893	A		7	$\frac{8}{11}$ and $\frac{2}{11}$	2,300	
		B	7	$\frac{8}{11}$ and $\frac{2}{11}$		2,200
27.11.1893	A		8	$\frac{8}{13}$ and $\frac{2}{13}$	1,400	
9.12.1893	A		9	1·0 and 0·5	0	

The above tables show that *in the steam-sterilised Loch Katrine water, the typhoid bacilli again underwent no multiplication, but on the contrary steady decline, the last surviving individuals being, however, remarkably persistent. Thus the typhoid bacilli were still discoverable on* 9.12.1893, *or fifty-one days after their first introduction. This is a much longer survival than in the case either of the steam-sterilised Thames or deep well waters.*

In the Loch Katrine water, rendered sterile by filtration, the typhoid bacilli also survived much longer than in either the similarly treated Thames or deep well waters, the bacilli being still discovered on 27.11.1893, *or thirty-nine days after their first introduction.* The filter used in this case was a porous cylinder of infusorial earth as with the Thames and deep well waters. This result again confirms what I found in the previous series of experiments (see pp. 460, 464, 479), viz., that the typhoid bacilli persisted much longer in the porcelain-filtered Loch Katrine than in the porcelain-filtered Thames water.

Comparison of Thames, Loch Katrine, and Deep Well Waters.

Typhoid-infected Steam-sterilised Deep Well Water.

Dates on which plate cultivations were made.	Particular flask employed. Kept at rest.	Particular flask employed. Daily agitated.	Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies obtained from 1 c.c. of water. Flask kept at rest.	Number of colonies obtained from 1 c.c. of water. Flask daily agitated.
19.10.1893	Before subdivision		5	c.c. $\frac{5}{11}$ and $\frac{2}{11}$	10,300	
30.10.1893	A		6	$\frac{8}{11}$ and $\frac{2}{11}$	2,900	
"		B	6	$\frac{8}{13}$ and $\frac{2}{13}$		2,800
8.11.1893	A		6	$\frac{2}{3}$ and $\frac{1}{6}$	1,100	
"		B	6	$\frac{8}{13}$ and $\frac{2}{13}$		840
20.11.1893	A		15	$\frac{4}{5}$ and $\frac{1}{5}$	0	

Typhoid-infected Porcelain-filtered Deep Well Water.

Dates on which plate cultivations were made.	Particular flask employed. Kept at rest.	Particular flask employed. Daily agitated.	Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies obtained from 1 c.c. of water. Flask kept at rest.	Number of colonies obtained from 1 c.c. of water. Flask daily agitated.
19.10.1893	Before subdivision		5	$\frac{1}{2}$ and $\frac{1}{5}$	12,200	
30.10.1893	A		13	$\frac{8}{11}$ and $\frac{2}{11}$	0	
"		B	13	$\frac{8}{11}$ and $\frac{2}{11}$		0
8.11.1893	A		12	3·0 and 2·0	0	
"		B	12	3·0 and 2·0		0

From the above tables it is seen that *in the steam-sterilised deep well water also the typhoid bacilli were incapable of multiplication, and, on the contrary, underwent continuous decline in numbers; they were last discovered on* 8.11.1893, *or twenty days after their introduction, whilst on* 20.11.1893, *or after being in the water for thirty-two days, they were no longer discoverable by plate-cultivation.* In this connection it is particularly noteworthy that the typhoid bacilli were still discovered on 21.11.1893 in the unsterilised deep well water, *thus showing that in this deep well water their longevity was unaffected by the circumstance of whether the water was sterilised or unsterilised.* In the case of both the Thames and Loch Katrine waters, on the other hand, the longevity of the typhoid bacilli was much greater in the sterilised than in the unsterilised water.

This circumstance is particularly instructive and important, inasmuch as it was just in this typhoid-infected unsterilised deep well water that the water bacteria present multiplied most extensively. and yet this large multiplication of the common water forms did not prejudicially affect the typhoid bacilli.

This deep well water, on the other hand, is in the sterilised condition less favourable to the longevity of the typhoid bacilli than the sterilised Thames and Loch Katrine waters, for in these three steam-sterilised waters the introduced typhoid bacilli disappeared first in the deep well and last in the Loch Katrine water, their longevity in the steam-sterilised Thames water being greater than in the deep well and less than in the Loch Katrine water. (For further remarks on this behaviour see p. 517.)

In the deep well water sterilised by filtration through porous porcelain (in this case again infusorial earth), the typhoid bacilli again disappeared with remarkable promptitude, being no longer discoverable eleven days after their introduction.

In order to ascertain whether these waters sterilised by filtration through porous cylinders owed the rapid disappearance of the typhoid and coli bacilli which almost invariably occurred in them to the presence of any antiseptic substance possessing general bactericidal properties, the following experiment was made:—

The typhoid-infected porcelain-filtered deep well water referred to above, and in which the typhoid bacillus was proved to be extinct on 30.10.1893, and 8.11.1893 respectively (see Table, p. 505), was on 11.11.1893 treated with three drops of the unsterilised uninfected deep well water. These three drops of unsterile water must have contained about 3000 water bacteria, as calculated from the results of plate cultivation given in the table on p. 499, and, as the volume of filtered water to which these three drops were added was about 100 c.c., the latter must have acquired about thirty water bacteria per 1 c.c. by the addition.

This porcelain-filtered deep well water, to which the three drops of unsterile deep well water were thus added on 11.11.1893, was examined by plate cultivation on 14.11.1893, *or three days after the addition, and was then found to contain* 10,462 *bacteria per* 1 *c.c.;* it was again examined *on* 23.11.1893, *or twelve days after the addition, and then contained* 603,900 *bacteria. It is obvious, therefore, that in this same water in which the typhoid bacilli were destroyed with such remarkable rapidity, some at any rate of the common water bacteria present in the unsterile deep well water were able to multiply both to an enormous extent and with wonderful celerity.* This result dismisses, in my opinion, the last lurking suspicion which might still remain of any antiseptic substance having accidentally gained access to the water in the process of filtration through these porous cylinders.

Comparison of Thames, Loch Katrine, and Deep Well Waters. Examination by Phenol Broth-culture

Date and number of broth tube.	Water used for cultivation with phenol broth.	Volume of water taken, c.c.	Quantity of phenol solution added to 10 c.c. broth.	Remarks.
20.10.1893	*Thames.*			
(1)	Uninfected unsterilised (A).....	1·0	3 drops	Turbid in 24 hours. On plate cultivation obtained a number of large surface milk-drop colonies and small depth colonies, often lenticular in shape. These yielded bubbles in gelatine, coagulated milk, gave a strong growth on potatoes, but a negative indol reaction. *Therefore neither typhoid nor* B. coli communis.
(10)	,, ,, (A).....	,,	5 ,,	Did not go turbid.
(4)	Typhoid-infected unsterilised (A)	,,	3 ,,	Turbid in 24 hours. These were not submitted to plate cultivation, as there could be no doubt that *typhoid was present.*
(13)	,, ,, (A)	,,	5 ,,	Turbid in 48 hours.
(7)	Typhoid-infected steam sterilised (A)	4 drops	3 ,,	Turbid in 24 hours.
(16)	Typhoid-infected steam sterilised (A)	4 ,,	5 ,,	Turbid in 24 hours.
28.10.1893				
(19)	Uninfected unsterilised (A).....	1·0	3 drops	Turbid in 24 hours. Plate cultivations yielded colonies of various kinds, including liquefying ones, but none resembling typhoid.
(25)	,, ,, (A).....	,,	5 ,,	Turbid in 5 days.
(31)	,, ,, (B).....	,,	3 ,,	Turbid in 24 hours.
(37)	,, ,, (B).....	,,	5 ,,	Did not go turbid.
(22)	Typhoid-infected unsterilised (A)	,,	3 ,,	Turbid in 24 hours. Plate cultivations yielded liquefying colonies (probably *B. liquidus*), but also numerous typical surface expansion colonies of typhoid bacillus. *Typhoid present.*
(28)	,, ,, (A)	,,	5 ,,	Did not go turbid.
(34)	,, ,, (B)	,,	3 ,,	Turbid in 24 hours. Plate cultivations yielded principally colonies causing liquefaction (probably *B. liquidus*), no colonies resembling typhoid. *Typhoid absent.*
(40)	,, ,, (B)	,,	5 ,,	Did not go turbid.

1.11.1893				
(43)	Uninfected unsterilised (A).....	1·0	3 drops	Turbid in 48 hours. Plate cultures gave principally liquefying colonies (probably *B. liquidus*), nothing like typhoid.
(49)	,, ,, (A).....	,,	5 ,,	Did not go turbid.
(55)	,, ,, (B).....	,,	3 ,,	Turbid in 48 hours. Same results as with No. 43 above.
(61)	,, ,, (B).....	,,	5 ,,	Did not go turbid.
(46)	Typhoid-infected unsterilised (A)	,,	3 ,,	Turbid in 24 hours. Plate cultures gave only liquefying colonies, nothing like typhoid. *Typhoid absent.*
(52)	,, ,, (A)	,,	5 ,,	Turbid in 72 hours. Plate cultures gave only very large thick milk-drop expansion colonies, giving bubbles in gelatine, but negative indol reaction. *Typhoid absent.*
(58)	,, ,, (B)	,,	3 ,,	Turbid in 24 hours Plate cultures gave only liquefying colonies and milk-drop colonies, nothing like typhoid. *Typhoid absent.*
(64)	,, ,, (B)	,,	5 ,,	Turbid in 72 hours. Plate cultures gave only milk-drop colonies, with faint resemblance to typhoid, but yielded bubbles in gelatine, negative indol, however. *Typhoid absent.*
7.11.1893				
(76)	Uninfected unsterilised (A).....	1·0	3 drops	Turbid in 48 hours.
(82)	,, ,, (A).....	,,	5 ,,	Turbid in 48 hours. Plate cultures contained characteristic colonies with thickened rim and clearer centre, not like typhoid.
(88)	,, ,, (B).....	,,	3 ,,	Turbid in 48 hours. Plate cultures contained only liquefying colonies (probably *B. liquidus*).
(94)	,, ,, (B).....	,,	5 ,,	Did not go turbid.
(79)	Typhoid-infected unsterilised (A)	,,	3 ,,	Turbid in 24 hours. Plate cultures contained only liquefying colonies (probably *B. liquidus*). *Typhoid absent.*
(85)	,, ,, (A)	,,	5 ,,	Did not go turbid.
(91)	,, ,, (B)	,,	3 ,,	Turbid in 24 hours. Results similar to those with No. 79. *Typhoid absent.*
(97)	,, ,, (B)	,,	5 ,,	Turbid in 48 hours. Plate cultures exhibited some surface colonies something like typhoid, but yellower; these gave bubbles in gelatine, but negative indol reaction. *Typhoid absent.*

Comparison of Thames, Loch Katrine, and Deep Well Waters. Examination by Phenol Broth-culture.

Date and number of broth tube.	Water used for cultivation with phenol broth.	Volume of water taken, c.c.	Quantity of phenol solution added to 10 c.c. broth.	Remarks.
20.10.1893	*Loch Katrine.*			
(3)	Uninfected unsterilised (A).....	1·0	3 drops.	Turbid in 24 hours.
(12)	,, ,, (A).....	,,	5 ,,	Did not go turbid.
(6)	Typhoid-infected unsterilised (A)	,,	3 ,,	Turbid in 24 hours. No plate cultures were made, as there could be no doubt as to presence of typhoid. *Typhoid present.*
(15)	,, ,, (A)	,,	5 ,,	Turbid in 24 hours.
(9)	Typhoid-infected steam sterilised (A)	,,	3 ,,	Turbid in 24 hours.
(18)	Typhoid-infected steam sterilised (A)	,,	5 ,,	Turbid in 24 hours.
28.10.1893				
(21)	Uninfected unsterilised (A).....	1·0	3 drops	Turbid in 24 hours. Plates contained only liquefying colonies (probably *B. liquidus*), nothing resembling typhoid.
(27)	,, ,, (A).....	,,	5 ,,	Did not go turbid.
(33)	,, ,, (B).....	,,	3 ,,	Turbid in 72 hours.
(39)	,, ,, (B).....	,,	5 ,,	Did not go turbid.
(24)	Typhoid-infected unsterilised (A)	,,	3 ,,	Turbid in 24 hours. Plates yielded the typical surface expansion colonies, which were proved to be typhoid by the usual tests. *Typhoid present.*
(30)	,, ,, (A)	,,	5 ,,	Did not go turbid.
(36)	,, ,, (B)	,,	3 ,,	Turbid in 24 hours.
(42)	,, ,, (B)	,,	5 ,,	Turbid in 24 hours. Plates exhibited a pure cultivation of typhoid. *Typhoid present.*

1.11.1893				
(45)	Uninfected unsterilised (A).....	1·0	3 drops	Did not go turbid.
(51)	" " (A).....	"	5 "	Did not go turbid.
(57)	" " (B).....	"	3 "	Did not go turbid.
(63)	" " (B).....	"	5 "	Did not go turbid.
(48)	Typhoid-infected unsterilised (A)	"	3 "	Turbid in 48 hours. Plates yielded pure cultivation of typhoid, confirmed by usual tests. *Typhoid present.*
(54)	" " (A)	"	5 "	Did not go turbid.
(60)	" " (B)	"	3 "	Turbid in 48 hours.
(66)	" " (B)	"	5 "	Turbid in 48 hours. Plates yielded pure cultivation of typhoid, confirmed by usual tests. *Typhoid present.*
7.11.1893				
(78)	Uninfected unsterilised (A).....	1·0	3 drops	Did not become turbid.
(84)	" " (A).....	"	5 "	Did not become turbid.
(90)	" " (B).....	"	3 "	Did not become turbid.
(96)	" " (B).....	"	5 "	Did not become turbid.
(81)	Typhoid-infected unsterilised (A)	"	3 "	Turbid in 48 hours.
(87)	" " (A)	"	5 "	Turbid in 48 hours. Plates gave a pure cultivation of typhoid, confirmed by usual tests. *Typhoid present.*
(93)	" " (B)	"	3 "	Turbid in 24 hours. Plates gave a pure cultivation of typhoid, confirmed by usual tests. *Typhoid present.*
(99)	" " (B)	"	5 "	Did not become turbid.
21.11.1893				
(126)	Uninfected unsterilised (A).....	1·0	3 drops	Did not become turbid.
(130)	" " (A).....	"	5 "	Did not become turbid.
(127)	Typhoid-infected unsterilised (A)	"	3 "	Turbid in 48 hours. Plates gave pink liquefying colonies (very like *B. prodigiosus*), nothing like typhoid. *Typhoid absent.*
(131)	" " (A)	"	5 "	Did not become turbid.
27.11.1893				
(160)	Uninfected unsterilised (A).....	1·0	3 drops	Turbid in 48 hours. Plates completely liquefied.
(164)	" " (A).....	"	5 "	Did not become turbid.
(161)	Typhoid-infected unsterilised (A)	"	3 "	Turbid in 48 hours. Plates contained pink liquefying colonies (like *B. prodigiosus*), nothing like typhoid on plate. *Typhoid absent.*
(165)	" " (A)	"	5 "	Did not become turbid.

Comparison of Thames, Loch Katrine, and Deep Well Waters. Examination by Phenol Broth-culture—*continued*.

Date and number of broth tube.	Water used for cultivation with phenol broth.	Volume of water taken, c.c.	Quantity of phenol solution added to 10 c.c. broth.	Remarks.
	Loch Katrine—cont.			
4.12.1893 (184)	Uninfected unsterilised (A).....	1·0	3 drops	Not turbid in five days. *Typhoid absent.*
(195)	" " (A).....	"	5 "	
(185)	Typhoid-infected unsterilised (A)	"	3 "	
(196)	" " (A)	"	5 "	
7.12.1893 (206)	Typhoid-infected unsterilised (A)	6·0	3 "	Turbid in 48 hours. Plates contained pink liquefying colonies as above, no surface colonies like typhoid; some depth colonies which gave bubbles in gelatine, but no indol reaction. *Typhoid absent.*

Comparison of Thames, Loch Katrine, and Deep Well Waters. Examination by Phenol Broth-culture.

Date and number of broth tube.	Water used for cultivation with phenol broth.	Volume of water taken in c.c.	Quantity of phenol solution added to 10 c.c. broth.	Remarks.
20.10.1893	*Deep Well Water (Kent).*			
(2)	Uninfected unsterilised (A).....	1·0	3 drops	Turbid in 48 hours. Plates contained pinhead and small milk-drop colonies, which gave bubbles in gelatine, coagulated milk, but yielded no indol.
(11)	,, ,, (A).....	,,	5 ,,	Did not become turbid.
(5)	Typhoid-infected unsterilised (A)	,,	3 ,,	Turbid in 24 hours. No plate cultures made from these, as of course at this stage *typhoid present.*
(14)	,, ,, (A)	,,	5 ,,	,, ,,
(8)	Typhoid-infected steam-sterilised (A)	4 drops	3 ,,	Turbid in 24 hours.
(17)	Typhoid-infected steam-sterilised (A)	,,	5 ,,	
28.10.1893				
(20)	Uninfected unsterilised (A).....	1·0	3 drops	Turbid in 72 hours.
(26)	,, ,, (A).....	,,	5 ,,	Did not become turbid.
(32)	,, ,, (B).....	,,	3 ,,	Turbid in 48 hours. Plates contained small smooth-rimmed depth colonies giving rise to very small milk-drop expansions not like typhoid.
(38)	,, ,, (B).....	,,	5 ,,	Did not become turbid.
(23)	Typhoid-infected unsterilised (A)	,,	3 ,,	Turbid in 24 hours. The majority of the colonies on the plates were liquefying (probably *B. liquidus*), but also a number of small colonies which may be typhoid.
(29)	,, ,, (A)	,,	5 ,,	Turbid in 72 hours. Plates contained large number of milk-drop colonies, but also some typical typhoid colonies. *Typhoid present.*
(35)	,, ,, (B)	,,	3 ,,	Turbid in 24 hours.
(41)	,, ,, (B)	,,	5 ,,	Turbid in 24 hours. These plates also gave a mixture of milk-drop and typical typhoid colonies, the latter were confirmed by usual tests. *Typhoid present.*

Comparison of Thames, Loch Katrine, and Deep Well Waters. Examination by Phenol Broth-culture—*continued*.

Date and number of broth tube.	Water used for cultivation with phenol broth.	Volume of water taken in c.c.	Quantity of phenol solution added to 10 c.c. broth.	Remarks.
1.11.1893	*Deep Well Water* (*Kent*)—cont.			
(44)	Uninfected unsterilised (A).....	1·0	3 drops	Turbid in 72 hours. Plates contained only colonies liquefying gelatine (apparently *B. liquidus* and *B. fluorescens liquefaciens*).
(50)	" " (A).....	"	5 "	Did not become turbid.
(56)	" " (B).....	"	3 "	Turbid in 72 hours. Plates contained only liquefying colonies (apparently *B. liquidus*).
(62)	" " (B).....	"	5 "	Did not become turbid.
(47)	Typhoid-infected unsterilised (A)	"	3 "	Turbid in 24 hours. Plates contained well-defined typhoid colonies besides numerous liquefying colonies. *Typhoid present.*
(53)	" " (A)	"	5 "	Turbid in 48 hours. Plates contained principally large milk-drop colonies, but a few typhoid colonies also discernible, and these confirmed by usual tests. *Typhoid present.*
(59)	" " (B)	"	3 "	Turbid in 24 hours.
(65)	" " (B)	"	5 "	Turbid in 24 hours. Plates again contained principally large milk-drop colonies, but also some typical typhoid colonies, which were confirmed by usual tests. *Typhoid present.*
				The organisms giving rise to these milk-drop colonies appear to grow more readily in the phenol broth than the typhoid bacillus, hence the latter was more easily discoverable in the tubes which had only received 3 drops of phenol.
7.11.1893				
(77)	Uninfected unsterilised (A).....	1·0	3 drops	Did not become turbid.
(83)	" " (A).....	"	5 "	Did not become turbid.
(89)	" " (B).....	"	3 "	Turbid in 48 hours. Plates contained fluorescent liquefying colonies, nothing like typhoid.
(95)	" " (B).....	"	5 "	Did not become turbid.
(80)	Typhoid-infected unsterilised (A)	"	3 "	Turbid in 24 hours. Plates contained both milk-drop and typical typhoid colonies, the latter confirmed by usual tests. *Typhoid present.*
(86)	" " (A)	"	5 "	Turbid in 48 hours. Plates contained only the milk-drop colonies.
(92)	" " (B)	"	3 "	Turbid in 24 hours. Plates contained both liquefying, milk-drop, and typical typhoid colonies, the latter confirmed by usual tests. *Typhoid present.*
(98)	" " (B)	"	5 "	Turbid in 48 hours. Plates contained both milk-drop and typical typhoid colonies. *Typhoid present.*

21.11.1893				
(124)	Uninfected unsterilised (A).....	1·0	3 drops	Turbid in 48 hours. Plates contained almost only fluorescent liquefying colonies. Nothing resembling typhoid.
(128)	,, ,, (A).....	,,	5 ,,	Did not become turbid.
(125)	Typhoid-infected unsterilised (A)	,,	3 ,,	Turbid in 24 hours. Plates contained, besides liquefying colonies, also a very large number of milk-drop colonies, but none resembling typhoid.
(129)	,, ,, (A)	,,	5 ,,	Turbid in 48 hours. Plates contained a large number of thick surface colonies which gave bubbles in gelatine, but no indol in broth. Also one typical typhoid colony which was confirmed by usual tests. *Typhoid present.*
27.11.1893				
(158)	Uninfected unsterilised (A)	1·0	3 drops	Did not become turbid.
(162)	,, ,, (A).....	,,	5 ,,	
(159)	Typhoid-infected unsterilised (A)	,,	3 ,,	Turbid in 24 hours.
(163)	,, ,, (A)	,,	5 ,,	Turbid in 48 hours. The plates contained, besides liquefying colonies, also some surface expansion colonies somewhat like typhoid but rather too thick, and these all gave bubbles in gelatine, although no indol reaction in broth. *Typhoid absent.*
4.12.1893				
(182)	Uninfected unsterilised (A).....	1·0	3 drops	Did not become turbid.
(193)	,, ,, (A).....	,,	5 ,,	
(183)	Typhoid-infected unsterilised (A)	,,	3 ,,	Turbid in 24 hours. Plates contained an apparently pure culture of the organism producing thick milk-drop colonies, and giving as usual bubbles in gelatine, but no indol reaction. *Typhoid absent.*
(194)	,, ,, (A)	,,	5 ,,	Turbid in 48 hours. These plates again contained only the thick milk-drop colonies. *Typhoid absent.*

The above three tables are of especial importance, exhibiting as they do the different behaviour of the typhoid bacillus *taken from one and the same cultivation and in approximately the same numbers*, on being introduced into these three different kinds of water in their natural unsterilised condition.

From the first table it will be seen that *in the Thames water the typhoid bacilli were demonstrable on* 28.10.1893, i.e., *nine days after their introduction, but already four days later, and afterwards, all endeavours to discover the typhoid bacillus proved abortive.*

From the second table, on the other hand, it will be seen that *in the Loch Katrine water the presence of the typhoid bacillus was demonstrable on* 7.11.1893, i.e., *nineteen days after its introduction, whilst on* 21.11.1893, *or fourteen days later, and afterwards, it could no more be discovered.*

From the third table, again, it will be seen that *in the deep-well water the typhoid bacilli were easily discoverable on* 7.11.1893, i.e., *nineteen days after their introduction, and just discoverable on* 21.11.1893, *or thirty-three days after their introduction, whilst, six days later and thereafter, all attempts to demonstrate their presence proved fruitless.*

As regards the effect of agitation in these experiments, it appears that the agitation on the whole promoted the multiplication of the water bacteria in the unsterilised waters, whilst it somewhat accelerated the disappearance of the typhoid bacilli in the infected sterile waters. These results partially confirm those obtained by Professor Ray Lankester in some similar experiments described by him to the recent Royal Commission on the London Water Supply (Appendix, p. 455).

In his experiments 2 litres of sterilised river water were placed in two similar jars, and each was similarly infected with the typhoid bacillus. One of the jars was then syringed four times an hour for twelve hours, and, after an interval, for eight hours more. The other jar was left undisturbed in the dark. The syringed jars showed a very marked inferiority in the number of typhoid germs obtained on cultivation, amounting to a reduction of one-half.

In my experiments the difference between the waters kept at rest and those submitted to agitation was not nearly so marked, which may possibly be due to the different mode of agitation employed, and also to the fact that the waters were not examined so soon after the agitation, for it is quite possible that the combined effect of agitation and oxygenation makes itself felt more in the first instance than later on.

In the case of the infected unsterilised waters, again, there is considerable evidence that the agitation hastened the disappearance of the typhoid bacilli. *Thus in the case of the unsterile Thames water,*

the typhoid bacilli were discovered on 28.10.1893, *or eight days after infection in the flask which was kept at rest, whilst they were not demonstrable on the same day in the case of the flask which had been subjected to agitation.*

In the unsterile Loch Katrine water, again, although on 7.11.1893 typhoid bacilli were found both in the water which had been kept at rest and in that which had been agitated, yet the broth-tube with five drops of the phenol solution only became turbid in the case of the water which had remained at rest, thus tending to show that the typhoid bacilli were more numerous or in a more active condition in this latter water than in that which had been agitated.

From the experiments made with the sterilised Thames, Katrine, and deep well waters, it is evident that in none of these waters does the typhoid bacillus proliferate, the longevity of the bacilli being greatest in the Katrine and least in the deep-well water; on the other hand, in the unsterilised waters their longevity is decidedly greatest in the deep-well, and decidedly least in the Thames, water.

In the sterilised water the principal factor determining longevity would, therefore, appear to be the proportion of organic matter present, which is much greater in the Loch Katrine and Thames than in the deep well water; on the other hand, in the unsterilised water the factor determining longevity must be an entirely different one; and whatever it may be, it is more conspicuous in the case of the two surface (Thames and Loch Katrine) than in that of the subterranean (deep well) water. It is, as has already been frequently indicated, generally believed that the more rapid disappearance of pathogenic bacteria in unsterile than in sterile waters is due to the multiplication or competition of the common water bacteria in the unsterile waters; but these experiments clearly show that this cannot be true, at any rate without some qualification, for by reference to the tables on pp. 495, 497, and 499, it will be seen that it was precisely in the case of the deep well water that the most extensive multiplication of the water bacteria took place. In this connection it is interesting to compare the following statement made by Professor Ray Lankester, F.R.S., to the recent Royal Commission on the London Water Supply (Appendix, p. 458) :—

"I took pure cultures of a very active and common fluviatile form, *B. fluorescens liquefaciens*, which suggested, by its vigorous action on gelatine, the possession of destructive properties. With this I mixed a pure culture of *Bacillus typhosus*, and studied the mixed culture, both by drop culture under the microscope and in the tube. During a fortnight no diminution of the activity or numbers of either species was observed. I have experimented with pure cultures of other fluviatile species, and intend to continue the observations. It is possible—indeed not improbable—that one or more species may be

discovered which are injurious to, or destructive of, *B. typhosus*, but I have not yet succeeded in establishing the fact."

It naturally suggests itself that possibly the particular kinds of water bacteria present in the three different kinds of water experimented with may have influenced the relative longevity of the typhoid bacillus in these three waters respectively. With a view to putting this supposition to the test, the following series of experiments were made:—

(*a*.) One portion of the typhoid-infected steam-sterilised Thames water was inoculated with a few drops of unsterilised Thames water, a second portion with a few drops of unsterilised Loch Katrine water, and a third portion with a few drops of unsterilised deep well water.

(*b*.) One portion of the typhoid-infected steam-sterilised Loch Katrine water was inoculated with a few drops of unsterilised Thames water, a second portion with a few drops of unsterilised Loch Katrine water, and a third portion with a few drops of unsterilised deep well water.

(*c*.) One portion of the typhoid-infected steam-sterilised deep well water was inoculated with a few drops of unsterilised Thames water, a second portion with a few drops of unsterilised Loch Katrine water, and a third portion with a few drops of unsterilised deep well water.

Unfortunately, this series of experiments was only commenced on 16.11.1893, when the number of typhoid bacilli in the three steam-sterilised waters had got very low (see Tables, pp. 501, 503, 505). In all instances there was an enormous multiplication of the water bacteria introduced in the few drops of unsterilised water added in each case, but owing to the small number of typhoid bacilli present, it was not possible to establish whether the rate of their disappearance was differently affected by the multiplication of the water bacteria from the unsterile Thames, Loch Katrine, or deep well water respectively.

Owing to the failure of the above series of experiments to determine whether the undoubtedly greater bactericidal properties of the unsterilised over the sterilised waters are due to the multiplication of the contained water bacteria, or to some other cause, the following fresh series of experiments was conducted on Thames water alone with the object of elucidating the same point.

Experiments on the Relative Longevity of the Typhoid Bacillus in Unsterilised Thames Water and in Steam-sterilised Thames Water Reinoculated with Thames Water Bacteria. (11.1.1894.)

In this series of experiments one portion of a sample of Thames water collected at Hampton on January 9th, 1894, was infected with typhoid in the natural unsterile condition, a second portion was similarly infected after having been previously sterilised by steaming, and a portion of the latter was again supplied with the Thames water bacteria by inoculating it with a few drops of the same unsterilised Thames water.

Infection of the Waters.—I have found in the previous series of experiments that, in taking the surface-growth from a well-matured typhoid culture, a large proportion of the bacilli are dead, whilst even those which are alive are often in a more or less weakened state. On this account I now generally prefer to take the bacilli for experiments such as these directly from fresh plate cultivations, selecting the largest and most vigorous looking colonies.

In the present series of experiments a gelatine plate culture (five days old) of the typhoid bacillus was employed; fifty-seven surface colonies were carefully removed by means of a sterile platinum loop and transferred to 50 c.c. of steam-sterilised Thames water in a small sterile-stoppered bottle; this was then violently shaken for fifteen minutes as usual, measured quantities of this liquid being then used for the infection of the large volumes of water. Thus, 800 c.c. of steam-sterilised Thames water were infected with 8 c.c. of the above liquid, 400 c.c. of this was kept, and will be referred to in the following experiments as

"*Typhoid-infected Steam-sterilised Thames Water.*"

To the remaining 400 c.c. was added 1 c.c. of the unsterilised Thames water, with the object of imparting to it the several kinds of water bacteria in Thames water. This portion will be referred to in the following experiments as

"*Typhoid-infected Steam-sterilised Thames Water inoculated with a few drops of Unsterile Thames Water.*"

Again, 400 c.c. of unsterilised Thames water were inoculated with 4 c.c. of the water attenuation of typhoid mentioned above, and this will be referred to in the following pages as

"*Typhoid-infected Unsterilised Thames Water.*"

These several infected waters, as well as some of the uninfected unsterilised Thames water, were placed in sterile flasks covered with

sterile beakers, and kept in a dark cupboard at a temperature of 9—11° C. All of these waters were periodically submitted to plate cultivation, as well as tested for typhoid bacilli by the method of phenol broth-culture. The results of these examinations are recorded in the following tables :—

Uninfected Unsterilised Thames Water (16.1.1894).

Dates on which plate cultivations were made.	Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies obtained from 1 c.c. of water.	Remarks.
16.1.1894	4	c.c. $\frac{1}{50}$ and $\frac{1}{100}$	5250	Only a very small number of liquefying colonies.
22.1.1894	8	$\frac{1}{100}$	5000	Ditto.
29.1.1894	8	$\frac{1}{50}$ and $\frac{1}{100}$	3750	Ditto.
5.2.1894	4	$\frac{1}{60}$ and $\frac{1}{120}$	2825	Ditto.
12.2.1894	7	$\frac{1}{50}$ and $\frac{1}{100}$	5500	No liquefying colonies.
19.2.1894	5	$\frac{1}{60}$ and $\frac{1}{120}$	3375	Ditto.
24.2.1894	5	$\frac{1}{50}$ and $\frac{1}{100}$	3750	Very few liquefying colonies only.

The above table shows that the sample of Thames water employed contained a considerable total number of bacteria, but of these unusually few caused liquefaction of the gelatine. Moreover, the water was characterised by the number of bacteria remaining practically stationary during the period (five weeks) over which these experiments extended, a phenomenon which I have already pointed out is not unfrequently met with in the case of surface waters.

Typhoid-infected Unsterilised Thames Water (16.1.1894).

Dates on which plate cultivations were made.	Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies obtained from 1 c.c. of water.	Remarks.
16.1.1894	4	c.c. $\frac{1}{60}$ and $\frac{1}{120}$	175,000	The plates contained only very few liquefying colonies, and an immense number of small colonies (typhoid, of course).
22.1.1894	4	$\frac{1}{60}$ and $\frac{1}{120}$	58,000	No liquefying colonies.
29.1.1894	4	$\frac{1}{60}$ and $\frac{1}{120}$	36,000	Some liquefying colonies, but also a number of typical typhoid surface expansion colonies, and a number of small depth colonies which may also be typhoid.
5.2.1894	4	$\frac{1}{50}$ and $\frac{1}{100}$	29,000	A few liquefying colonies, numerous small depth colonies, but no typical surface expansion typhoid colonies.
12.2.1894	5	$\frac{1}{120}$	6,000	Ditto.
19.2.1894	4	$\frac{1}{50}$ and $\frac{1}{100}$	6,300	Ditto.
24.2.1894	5	$\frac{1}{50}$ and $\frac{1}{100}$	6,700	Ditto.

Bearing in mind (see table, p. 520) that the uninfected unsterilised Thames water contained about 5000 water bacteria in 1 c.c. on the day of infection, it is evident that about 170,000 typhoid bacilli per c.c. were introduced. The periodical examinations recorded above show that these numbers steadily declined, and from the character of the colonies obtained on the plates, it appears that the water bacteria underwent no marked multiplication, thus, no increase in the number of liquefying colonies was observed. The gradual diminution in the total number of colonies obtained may be ascribed, therefore, to the dying off of the 170,000 typhoid bacilli per c.c. introduced (see also results of phenol broth-culture experiments, p. 525, *et seq.*).

Typhoid-infected Steam-sterilised Thames Water (16.1.1894).

Dates on which plate cultivations were made.	Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies obtained from 1 c.c. of water.	Remarks.
16.1.1894	4	c.c. $\frac{5}{9}$ and $\frac{2}{9}$	103,000	Pure cultivation of typhoid.
22.1.1894	6	$\frac{1}{5}$ and $\frac{1}{10}$	129,000	Ditto. The increase in number is so slight that it is not attributable to multiplication but rather to the breaking up of aggregates, and possibly also to the longer incubation of the plates.
29.1.1894	..	..	..	Plates accidentally lost.
5.2.1894	5	$\frac{2}{11}$ and $\frac{1}{11}$	76,500	Pure cultivation of typhoid, but the surface colonies have only very slightly expanded owing to the plate being much crowded.
12.2.1894	3	$\frac{1}{6}$ and $\frac{1}{12}$	64,600	Ditto.
19.2.1894	5	$\frac{1}{5}$ and $\frac{1}{10}$	17,080	Ditto.
24.2.1894	5	$\frac{2}{9}$ and $\frac{1}{9}$	5,337	Ditto.

As usual, then, in the steam-sterilised water, the typhoid bacilli underwent a gradual decline, the slight increase on 22.1.1894 being, in my opinion, not attributable to real multiplication, but to other causes as indicated above. The typhoid bacilli were still abundantly present thirty-nine days after their first introduction. I attribute this greater longevity in this series of experiments partly to the fact that the typhoid bacilli were initially introduced in such large numbers, and partly to their doubtless being in a very vigorous condition, having been taken from colonies selected for their size, and only five days old at the time.

Typhoid-infected Steam-sterilised Thames Water inoculated with a few drops of Unsterile Thames Water (16.1.1894).

Dates on which plate cultivations were made.	Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies obtained from 1 c.c. of water.	Remarks.
		c.c.		
16.1.1894	4	$\frac{1}{2}$ and $\frac{1}{5}$	115,000	Practically pure cultivation of typhoid.
22.1.1894	3	$\frac{1}{5}$ and $\frac{1}{10}$	116,500	Very large number of fluorescent liquefying colonies, also a large number of surface expansion colonies which may be typhoid, and a large number of small depth colonies.
29.1.1894	3	$\frac{1}{50}$ and $\frac{1}{100}$	44,875	Numerous liquefying colonies, numerous small depth colonies, only a few typical surface expansion colonies resembling typhoid. On the same day the presence of typhoid was *far* more conspicuous on the plates of the typhoid-infected unsterilised water (see Table, p. 521).
5.2.1894	4	$\frac{1}{100}$	7,200	Ditto.
12.2.1894	4	$\frac{1}{100}$	5,200	Ditto.
19.2.1894	3	$\frac{1}{50}$ and $\frac{1}{100}$	2,350	Numerous liquefying colonies, and depth colonies, but no typical surface expansions like typhoid, although of course the depth colonies *may* be typhoid.
24.2.1894	4	$\frac{1}{50}$ and $\frac{1}{100}$	36,600	Ditto.
7.3.1894	5	$\frac{1}{100}$	18,500	Ditto.

The 115,000 bacteria per 1 c.c. contained in this water must have been almost exclusively typhoid bacilli, for, after infection with typhoid, 400 c.c. were inoculated with 1 c.c. of unsterile Thames water. Now, this unsterile water, as seen from table, p. 520, contained about 5000 water bacteria per 1 c.c.; 5000 water bacteria must thus have been added to 400 c.c. of this typhoid-infected steam sterilised Thames water, which would thus contain about twelve water bacteria per 1 c.c. in addition to the typhoid bacilli with which it had been previously infected. These few water bacteria must have undergone rapid and extensive multiplication, for, on 22.1.1894, a

very large number of fluorescent liquefying colonies was found on the plates, and in all the subsequent examinations of this water numerous liquefying colonies were also present, thus clearly showing that the diminution in the total number of colonies found in the successive plates must have been in large measure due to the dying off of the typhoid bacilli.

Thus, whilst in the case of the simply typhoid-infected unsterilised Thames water there is no evidence (see table, p. 521) of multiplication of the contained water bacteria having taken place, in this typhoid-infected steam-sterilised Thames water, to which a few water bacteria had been added, there is evidence of abundant multiplication of these water bacteria having occurred, and it becomes a matter of the greatest importance to ascertain in which of these two waters the typhoid bacilli (present in each case in approximately the same numbers and having exactly the same origin and history) exhibited the greater longevity. This question was determined by means of the examinations by phenol broth-culture, the results of which are recorded in the following tables:—

Thames Water (series of Experiments begun 16.1.1894). Examinations by Phenol Broth-culture.

Date and number of broth tube.	Water used for cultivation with phenol broth.	Volume of water taken in c.c.	Quantity of phenol solution added to 10 c.c. broth.	Remarks.
22.1.1894.	*Thames Water.*			
(304)	Unsterilised uninfected	1·0	3 drops	Turbid in 24 hours. Plate cultivations exhibited a pure cultivation of what was apparently *B. liquidus* (Percy Frankland), *nothing resembling typhoid colonies on the plates.*
(308)	Ditto	,,	5 ,,	Did not become turbid.
(305)	Typhoid-infected unsterilised	,,	3 ,,	Turbid in 24 hours.
(309)	Ditto	,,	5 ,,	Turbid in 24 hours. Plates gave typhoid surface expansion and depth colonies, confirmed by negative milk, negative indol, potatoes, and negative bubbles as usual. *Typhoid present.*
(302)	Typhoid-infected steam-sterilised	,,	3 ,,	Turbid in 24 hours. } No plates poured, as, of course, *typhoid present.*
(306)	Ditto	,,	5 ,,	Turbid in 24 hours. }
(303)	Typhoid-infected steam-sterilised inoculated with few drops of unsterile Thames	,,	3 ,,	Turbid in 24 hours.
(307)	Ditto	,,	5 ,,	Turbid in 24 hours. Plates gave typical typhoid colonies, confirmed by usual tests. *Typhoid present.*
29.1.1894.				
(314)	Unsterilised uninfected	1·0	3 drops	Turbid in 48 hours. Plates exhibited a pure cultivation of what was apparently *B. liquidus, nothing resembling typhoid colonies on the plates.*
(318)	Ditto	,,	5 ,,	Did not become turbid.
(315)	Typhoid-infected unsterilised	,,	3 ,,	Turbid in 24 hours. } Plates from both gave typical typhoid colonies, confirmed by usual tests. *Typhoid present.*
(319)	Ditto	,,	5 ,,	Turbid in 24 hours. }
(312)	Typhoid-infected steam-sterilised	,,	3 ,,	Turbid in 24 hours. Plates gave typical typhoid colonies, confirmed by usual tests. *Typhoid present.*
(316)	Ditto	,,	5 ,,	Turbid in 24 hours.
(313)	Typhoid-infected steam-sterilised inoculated with few drops of unsterile Thames	,,	3 ,,	Turbid in 24 hours. Plates gave typical typhoid colonies, confirmed by usual tests. *Typhoid present.*
(317)	Ditto	,,	5 ,,	Turbid in 48 hours.

Thames Water (series of Experiments begun 16.1.1894). Examinations by Phenol Broth culture—*continued.*

Date and number of broth tube.	Water used for cultivation with phenol broth.	Volume of water taken in c.c.	Quantity of phenol solution added to 10 c.c. broth.	Remarks.
5.2.1894.	*Thames Water*—cont.			
(322)	Unsterilised uninfected	1·0	3 drops	Turbid in 48 hours. Plates again exhibited a pure cultivation of what was apparently *B. liquidus, nothing resembling typhoid colonies on the plates.*
(326)	Ditto	,,	5 ,,	Did not become turbid.
(323)	Typhoid-infected unsterilised	,,	3 ,,	Turbid in 24 hours. } Plates from both gave typical typhoid colonies, confirmed by usual tests. *Typhoid present.*
(327)	Ditto	,,	5 ,,	Turbid in 48 hours. }
(320)	Typhoid-infected steam-sterilised	,,	3 ,,	Turbid in 24 hours. } Plates from both gave typical typhoid colonies, confirmed by usual tests. *Typhoid present.*
(324)	Ditto	,,	5 ,,	Turbid in 24 hours. }
(321)	Typhoid-infected steam-sterilised inoculated with few drops of unsterile Thames	,,	3 ,,	Turbid in 24 hours. } Plates from both gave typical typhoid colonies, confirmed by usual tests. *Typhoid present.*
(325)	Ditto	,,	5 ,,	Turbid in 48 hours. }
12.2.1894.				
(332)	Unsterilised uninfected	1·0	3 drops	} Not turbid after 5 days.
(336)	Ditto	,,	5 ,,	}
(333)	Typhoid-infected unsterilised	,,	3 ,,	} Not turbid after 5 days. *Typhoid absent.*
(337)	Ditto	,,	5 ,,	}
(330)	Typhoid-infected steam-sterilised	,,	3 ,,	Turbid in 24 hours. } No plates poured, but, of course, *typhoid present.*
(334)	Ditto	,,	5 ,,	Turbid in 48 hours. }
(331)	Typhoid-infected steam-sterilised inoculated with few drops of unsterile Thames	,,	3 ,,	Turbid in 24 hours. } Plates from both gave typical typhoid colonies, confirmed by usual tests. *Typhoid present.*
(335)	Ditto	,,	5 ,,	Turbid in 72 hours. }

15.2.1894.				
(350)	Uninfected unsterilised...........	1·0	3 drops	Did not become turbid.
(352)	Ditto........................	3·0	„	Turbid in 48 hours. Plates gave pure cultivation of what appeared to be *B. liquidus*. No colonies like typhoid.
(351)	Typhoid-infected unsterilised	1·0	„	Turbid in 48 hours. Plates gave pure cultivation of what appeared to be *B. liquidus*. No colonies like typhoid. *Typhoid absent.*
(353)	Ditto........................	3·0	„	Turbid in 48 hours. Plates gave large number of small colonies not unlike typhoid. On inoculation into gelatine, found to liquefy on long keeping. On potatoes gave diffused, colourless growth rather more conspicuous than typhoid, but still not sufficiently conclusive. Poured gelatine plates from potato culture, crowded plates had the gelatine softened, and the colonies which had not liquefied on the less crowded ones caused liquefaction on inoculating into gelatine and keeping. *Typhoid absent.*
19.2.1894.				
(360)	Unsterilised uninfected...........	1·0	3 drops	Did not become turbid.
(361)	Ditto........................	3·0	„	Turbid in 48 hours. Plates contained colonies of *B. liquidus* and small smooth-rimmed colonies; the latter, on inoculation into broth, rendered the latter turbid and formed a pellicle on the surface, therefore not typhoid.
(362)	Typhoid-infected unsterilised	1·0	„	Did not become turbid.
(363)	Ditto........................	3·0	„	Turbid in 48 hours. Plates gave a pure cultivation of what appeared to be *B. liquidus*, and contained nothing like typhoid colonies. *Typhoid absent.*
(358)	Typhoid-infected steam-sterilised..	1·0	„	Turbid in 24 hours. No plates poured, but, of course, *typhoid present.*
(359)	Typhoid-infected steam-sterilised inoculated with few drops of unsterile Thames...............	„	„	Turbid in 48 hours. Plates exhibited typical typhoid depth colonies, but many of the depth colonies were curiously lobulated, and had even corkscrew-like prolongations; fresh plates were poured from one of these depth colonies, and these contained only typical typhoid colonies, both depth and surface. *Typhoid present.*
26.2.1894.				
(380)	Typhoid-infected steam-sterilised..	1·0	3 drops	Turbid in 48 hours. No plates poured, but, of course, *typhoid present.*
(381)	Typhoid-infected steam-sterilised inoculated with few drops of unsterile Thames...............	„	„	Did not become turbid. *Typhoid absent.*
1.3.1894.				
(384)	Typhoid-infected steam-sterilised..	1·0	3 drops	Turbid in 24 hours. Plates gave typical typhoid colonies, confirmed by usual tests. *Typhoid present.*
(386)	Typhoid-infected steam-sterilised inoculated with few drops of unsterile Thames...............	„	„	Did not become turbid in 4 days.
(385)	Ditto........................	3·0	„	Turbid in 4 days. The plates gave a pure cultivation of what appeared to be *B. liquidus*, and contained nothing like typhoid colonies. *Typhoid absent.*
5.3.1894.				
(387)	Typhoid-infected steam-sterilised..	1·0	3 drops	Turbid in 48 hours. *Typhoid present.*
(388)	Ditto........................	3·0	„	

The above table clearly shows

(1.) That *the uninfected unsterilised Thames water* contained no bacteria which could be mistaken for typhoid bacilli by the methods of investigation employed. The phenol broth-tubes which became turbid on inoculation with this water were always found by plate cultivation to yield colonies which have all the appearance of the *B. liquidus* formerly described by me ('Zeitschrift für Hygiene,' vol. 6, 1889; 'Roy. Soc. Proc.,' vol. 53, 1893, p. 186). In all these experiments in which the method of phenol broth-culture has been employed, I have repeatedly found that this same organism succeeds in developing in the broth to which three drops of the phenol solution per 10 c.c. of broth are added.

(2.) That *the typhoid-infected steam-sterilised Thames water* contained living typhoid bacilli throughout the entire period of forty-eight days (January 16th—March 5th, 1894) over which this series of experiments extended.

(3.) That in *the typhoid-infected unsterilised Thames water*, the typhoid bacilli were still demonstrable on 5.2.1894, or twenty days after their introduction; but seven days later, on 12.2.1894, they were no longer discoverable.

(4.) That in *the typhoid-infected steam-sterilised Thames water, which had been furnished with the Thames water bacteria by inoculating with a few drops of unsterilised Thames water*, the typhoid bacilli were still demonstrable on 19.2.1894, or thirty-four days after their introduction, whilst seven days later, on 26.2.1894, they were no longer discoverable.

(5.) Thus, not only was the longevity of the typhoid bacilli far greater, as usual, in the sterilised than in the unsterile waters, but of the two unsterile waters, the one naturally so, and the other rendered unsterile by the inoculation of a few drops of unsterile Thames water, the naturally unsterile one proved to be decidedly more antagonistic to the vitality of the typhoid bacillus, than the water rendered artificially unsterile, as we may call it, by inoculation with unsterile Thames water.

(6.) This result is the more significant and important, inasmuch as it was shown (see p. 521) that in the naturally unsterile water no multiplication of the water bacteria took place, whilst, in what we may call the artificially unsterile water, it was shown (see p. 523) that a very large multiplication of the introduced water bacteria certainly did take place.

(7.) In the previous series of experiments (see p. 516) it was equally clearly shown that the typhoid bacilli enjoyed a greater longevity in the unsterile deep well water than in

either the unsterile Thames or Loch Katrine waters, although it was precisely in the deep well water that the water bacteria underwent multiplication.

(8.) These experimental observations lead me to the conclusion that the antagonistic action of the unsterile waters on the typhoid bacillus is not to be attributed to the multiplication of the water bacteria leading to the suppression of the typhoid bacilli "by competition in the struggle for existence," to use the common phraseology of many writers on these subjects, but through the existence in the unsterile waters of conditions (due, doubtless, to a great extent to the presence of chemical products elaborated by water bacteria) which are inimical to the vitality of the typhoid bacillus.

That conditions inimical to the vitality of the typhoid bacillus *can be generated by the water bacteria alone* is demonstrated by the above experiments in which a few drops of unsterile Thames water were added to the typhoid-infected steam-sterilised water, with the result that the longevity of the typhoid bacilli in this water was far less than in the steam-sterilised water itself.

That the longevity of the typhoid bacillus is still less in the naturally unsterile water than in that rendered unsterile by inoculation, I attribute to the fact that in the naturally unsterile Thames water, countless generations of water bacteria have flourished before the water is made the subject of experiment at all, and it must, therefore, be more or less saturated with those bacterial products which are prejudicial to the vitality of the typhoid bacillus, and which, in fact, frequently hamper or even inhibit the further multiplication of the water bacteria themselves.

The deep well water, on the other hand, in its natural condition is in a very different state; as my numerous former examinations (see Second Report, pp. 178—180) of this water have shown, it is drawn from its subterranean source in an almost perfectly sterile condition, having never since its exhaustive filtration through the porous strata of the earth, in which it has altogether altered its chemical composition, harboured any micro-organisms at all, so that the abundant bacterial multiplication, which, as I have shown, it exhibits in the laboratory, is really the first time that it is subjected to the influence of bacterial growth, and it takes a correspondingly longer time, therefore, for the conditions inimical to the typhoid bacillus to be established. In fact, in the experiments with deep well water (see pp. 505, 515) the typhoid bacillus was actually discovered in the unsterile water a little later than in the steam-sterilised water. That the multiplication of the bacteria in the deep well water does not lead to conditions so antagonistic to the vitality of the typhoid bacillus is also, no doubt, attri-

butable to the circumstance that the number of different kinds of bacteria in the deep well water is much more limited than in ordinary surface waters.

For the practical hygienic application of these experimental observations, reference should be made to the final conclusions at the end of this Report, see p. 543.

Further Experiments on the Influence of the Addition of Common Salt to Water containing the Typhoid Bacillus.

The several waters prepared for the last series of experiments were also made to serve the purpose of verifying the remarkable results previously obtained (see p. 434, *et seq.*) by the addition of common salt to typhoid-infected waters.

These experiments were commenced on 12.2.1894, on which day some of each of the following waters (for full particulars concerning them, see p. 519) received 1 per cent. of pure sterile sodium chloride respectively :—

(*a.*) *Typhoid-infected steam-sterilised Thames water.*
(*b.*) *Typhoid-infected steam-sterilised Thames water, inoculated with a few drops of unsterile Thames water.*
(*c.*) *Uninfected unsterilised Thames water.*
(*d.*) *Typhoid-infected unsterilised Thames water.*

These waters, to each of which 1 per cent. of sodium chloride had been added, were preserved in a dark cupboard at a temperature of 9—11° C., and were submitted to periodical examination along with the several waters of the last series, with which they were to be compared.

Uninfected Unsterilised Thames Water to which 1 per cent. of Sodium Chloride was added on 12.2.1894.

Dates on which plate cultivations were made.	Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies obtained from 1 c.c. of water.	Remarks.
14.2.1894	5	c.c. $\frac{1}{50}$ and $\frac{1}{100}$	2,150	Only very small number of liquefying colonies.
19.2.1894	3	$\frac{1}{50}$ and $\frac{1}{100}$	607,000	Very large number of liquefying colonies.
24.2.1894	3	$\frac{1}{50}$ and $\frac{1}{100}$	1,200,000	Numerous liquefying colonies.

On comparing this table with the one on p. 520, which refers to the same water, only without the addition of salt, it will be seen that the effect of the salt addition was to cause an enormous and rapid multiplication of the water bacteria. The liquefying colonies underwent great multiplication, but there was also an enormous increase in other non-liquefying colonies of various kinds.

Typhoid-infected Unsterilised Thames Water to which 1 per cent. of Sodium Chloride was added on 12.2.1894.

Dates on which plate cultivations were made.	Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies obtained from 1 c.c. of water.	Remarks.
14.2.1894	5	c.c. $\frac{1}{100}$	5,000	Only a very small number of liquefying colonies.
19.2.1894	3	$\frac{1}{80}$ and $\frac{1}{60}$	404,000	Considerable number of liquefying colonies, but special increase in small non-liquefying colonies.
24.2.1894	3	$\frac{1}{50}$ and $\frac{1}{100}$	407,000	Comparatively few liquefying colonies, but an enormous number of small non-liquefying colonies.

On comparing the above table with that on p. 521, it will be seen what an enormous multiplication of the bacteria present was caused by the addition of the salt; in this case the multiplication was not so much shared in by the liquefying organisms as by the others.

Typhoid-infected Steam-sterilised Thames Water to which 1 per cent. of Sodium Chloride was added on 12.2.1894.

Dates on which plate cultivations were made.	Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies obtained from 1 c.c. of water.	Remarks.
14.2.1894	5	c.c. $\frac{1}{5}$ and $\frac{1}{10}$	17,385	Typical pure cultivation of typhoid.
19.2.1894	8	$\frac{1}{5}$ and $\frac{1}{10}$	360	Ditto.
24.2.1894	16	$\frac{5}{6}$ and $\frac{5}{12}$	6	Only depth colonies on the plate.

On comparing this table with that on p. 522, it will be seen that the addition of the salt was highly prejudicial to the typhoid bacilli, the latter exhibiting a most rapid diminution in number, and degeneration in their vitality.

Typhoid-infected Steam-sterilised Thames Water inoculated with a few drops of Unsterile Thames Water, to this 1 per cent. of Sodium Chloride was added on 12.2.1894.

Dates on which plate cultivations were made.	Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies obtained from 1 c.c. of water.	Remarks.
14.2.1894	3	c.c. $\frac{1}{50}$ and $\frac{1}{100}$	25,725	Numerous liquefying colonies and large number of small depth colonies.
19.2.1894	2	$\frac{1}{60}$ and $\frac{1}{120}$	296,500	Ditto.
24.2.1894	3	$\frac{1}{50}$ and $\frac{1}{100}$	340,000	Ditto.

On referring to the table on p. 523, it will be seen that in this water, before the addition of the salt, a considerable multiplication of the water bacteria had taken place, but at the time the salt was added, the total number of bacteria only amounted to from 2000—5000 per 1 c.c.; after the addition of the salt, however, an enormous multiplication followed.

We must now see how the vitality of the typhoid bacilli was affected by this enormous bacterial multiplication which took place in the unsterile waters after the 1 per cent. of common salt was added to them. This was, of course, ascertained by the method of phenol broth-culture, the results of which are recorded in the following table :—

Thames Water with additions of 1 per cent. Common Salt (12.2.1894). Examinations by Phenol Broth-culture.

Date and number of broth tube.	Water used for cultivation with phenol broth.	Volume of water taken in c.c.	Quantity of phenol solution added to 10 c.c. broth.	Remarks.
14.2.1894	*Thames Water.*			
(340)	Uninfected unsterilised, 1 per cent. sodium chloride added, 12.2.1890	1·0	3 drops	Turbid in 48 hours. Plates gave a pure cultivation of what appeared to be *B. liquidus* (Percy Frankland). Nothing like typhoid colonies on plates.
(344)	Ditto	"	5 "	Not turbid in 5 days.
(341)	Typhoid-infected unsterilised, 1 per cent. sodium chloride added, 12.2 1894	"	3 "	Turbid in 48 hours. Plates gave a few liquefying and many depth colonies, some of which formed small expansions, not unlike typhoid. These found to coagulate milk, and to give greenish-yellow growth on potatoes, also liquefied gelatine slowly. *Not typhoid.*
(345)	Ditto	"	5 "	Not turbid in 5 days.
(338)	Typhoid-infected steam-sterilised, 1 per cent. sodium chloride added, 12.2.1894	"	3 "	Turbid in 24 hours. Plates gave typical typhoid colonies, confirmed by usual tests. *Typhoid present.*
(342)	Ditto	"	5 "	Turbid in 48 hours.
(339)	Typhoid-infected steam-sterilised, inoculated with few drops of unsterile Thames, 1 per cent. sodium chloride added, 12.2.1894	"	3 "	Turbid in 48 hours. Plates gave typical typhoid colonies, confirmed by usual tests. *Typhoid present.*
(343)	Ditto	"	5 "	Not turbid in 5 days.
19.2.1894 (366)	Uninfected unsterilised, 1 per cent. sodium chloride added, 12.2.1894	1·0	3 drops	Did not become turbid.
(367)	Typhoid-infected unsterilised, 1 per cent. sodium chloride added, 12.2.1894	"	3 "	Did not become turbid. *Typhoid absent.*
(364)	Typhoid-infected steam-sterilised, 1 per cent. sodium chloride added, 12.2.1894	"	3 "	Turbid in 48 hours. Plates gave numerous depth colonies, but only one typical typhoid expansion. Many of the depth colonies were much lobulated, and exhibited whip-like prolongations. Fresh plates were poured from one of these colonies, and these plates exhibited the typical typhoid colonies. *Typhoid present.*
(365)	Typhoid-infected steam-sterilised, inoculated with few drops of unsterile Thames, 1 per cent. sodium chloride added, 12.2.1894	"	3 "	Did not become turbid. *Typhoid absent.*

Thames Water with additions of 1 per cent. Common Salt (12.2.1894). Examinations by Phenol Broth-culture—*continued.*

Date and number of broth tube.	Water used for cultivation with phenol broth.	Volume of water taken in c.c.	Quantity of phenol solution added to 10 c.c. broth.	Remarks.
	Thames Water—cont.			
22.2.1894 (369)	Uninfected unsterilised, 1 per cent. sodium chloride added, 12.2.1894	3·0	3 drops	Turbid in 4 days. Plates gave a pure cultivation of what appeared to be *B. liquidus*. Nothing like typhoid colonies on the plates.
(370)	Typhoid-infected unsterilised, 1 per cent. sodium chloride added, 12.2.1894	,,	3 ,,	Turbid in 24 hours. Plates gave a pure cultivation of what appeared to be *B. liquidus*. Nothing like typhoid colonies on the plates. *Typhoid absent.*
(368)	Typhoid-infected steam-sterilised, inoculated with few drops of unsterile Thames, 1 per cent. sodium chloride added, 12.2.1894	,,	3 ,,	Turbid in 48 hours. Plates gave only liquefying colonies. *Typhoid absent.*
26.2.1894 (382)	Typhoid-infected steam-sterilised, 1 per cent. sodium chloride added, 12.2.1894	1·0	3 drops	Turbid in 5 days. Plates gave the same lobulated colonies noticed above (Broth tube No. 364). *Typhoid present.*
(383)	Typhoid-infected steam-sterilised, inoculated with few drops unsterile Thames, 1 per cent. sodium chloride added, 12.2.1894	3·0	3 ,,	Turbid in 48 hours. Plates gave only liquefying colonies. *Typhoid absent*
5.3.1894 (389)	Typhoid-infected steam-sterilised, 1 per cent. sodium chloride added, 12.2.1894	1·0	3 drops	Not turbid in 7 days. *Typhoid absent.*
(390)	Ditto	3·0	3 ,,	Not turbid in 7 days.

The above table should be compared with that on p. 525 *et seq.*, which refers to the same waters only without the addition of salt. It will then be seen :—

(1.) That *in the typhoid-infected unsterilised Thames water*, the typhoid bacilli had already disappeared on 12.2.1894, *i.e.*, before the addition of salt was made at all, and, therefore, that no inferences can be drawn as to the effect of the salt on the typhoid bacilli in this unsterilised water. Of course the absence of typhoid in this unsterile water was not ascertained until more than a week after this date, 12.2.1894, on which the salt was added, otherwise the experiment would not have been made at all.

(2.) *In the typhoid-infected steam-sterilised Thames water, inoculated with a few drops of unsterile Thames*, the typhoid bacilli were still demonstrable on 19.2.1894, but not on 26.2.1894, whilst in the same water, to which 1 per cent. of salt was added on 12.2.1894, the typhoid bacilli were discoverable still on 14.2.1894, *but not on 19.2.1894.* Thus the addition of the salt considerably hastened the disappearance of the typhoid bacilli from what may be called this artificially unsterile Thames water.

(3.) As already shown by the results of plate cultivation given on p. 531, the addition of the 1 per cent. of salt to the *typhoid-infected steam-sterilised Thames water*, caused a very rapid diminution in the number of typhoid bacilli present, and these results are entirely confirmed by the results on cultivation with phenol broth. Thus, the last plate cultivation, made on 24.2.1894, revealed the presence of only six typhoid bacilli in 1 c.c., phenol broth-culture still showed the presence of typhoid on 26.2.1894, but no more on 5.3.1894, although in the same water, which had not been treated with sodium chloride, the typhoid bacilli were easily discoverable by phenol broth-culture on that day.

(4.) There can be no doubt, therefore, that common salt, whilst enormously stimulating the multiplication of many forms of water bacteria, exerts a directly and highly prejudicial effect on the typhoid bacilli, causing their rapid disappearance from the water whether water bacteria are present or not.

(5.) It is worthy of remark that the typhoid colonies obtained on plate cultivation of the phenol-broth tubes which had been rendered turbid by these infected saline waters exhibited in some cases a very abnormal appearance, being irregularly swollen and lobulated in the depth and often giving rise to whip-like prolongations into the surrounding gelatine. As

far as I am aware, such abnormally formed typhoid colonies have not been previously observed, and I am inclined to attribute them to the degeneration of the typhoid bacilli, for, on passing such colonies through a further process of plate cultivation, only the normally formed typhoid colonies were obtained.

On the Possibility of the Typhoid Bacillus or the B. coli communis *multiplying in Potable Water.*

In none of the several series of experiments with Thames, Loch Katrine, and deep well (chalk) water, already recorded, was there any evidence of the typhoid bacillus undergoing any multiplication whatsoever; on the contrary, in all cases in which the typhoid bacillus was introduced into these waters in a sterilised condition there was a more or less rapid diminution in its numbers observed. In the case of the *B. coli communis*, in the steam-sterilised Thames water (p. 454), there was considerable multiplication of the *B. coli communis* observed when the water was kept at a summer, but practically none when it was maintained at the winter, temperature; on the other hand, in the steam-sterilised Loch Katrine water (see p. 481) the *B. coli communis* did not exhibit any numerical increase, but, on the contrary, rapid decline.

Of the waters experimented with above, the Thames water contains about the average quantity of organic matter present in those surface waters from cultivated land which are supplied for domestic purposes, whilst similarly the extremely small proportion of organic matter in the Kent well water is typical of the water supplied from deep wells; on the other hand, the Loch Katrine water contains decidedly less organic matter than is usually present in the water supplied to towns from upland surface sources. Now, although the experiments which have been detailed above conclusively show that the typhoid bacillus does not proliferate in the Thames, deep well, or Loch Katrine water employed, it appeared to me to be of importance to ascertain whether in upland surface water, more highly impregnated with organic matter than is the case with that from Loch Katrine, such proliferation of the typhoid bacillus might not perhaps take place.

For this purpose I employed, as specially fitted for the object in view, some moorland surface water which is supplied to a large manufacturing population in North Britain, and which the following analysis shows is very highly charged with organic matter of a peaty character:—

	Results of analysis expressed in parts per 100,000.
Total solid matter	8·80
Organic carbon (by combustion)	0·623
„ nitrogen („)	0·049
„ „ (by Kjeldahl process)	0·044
Ammonia (free)	0·0
„ (albuminoid)	0·023
Oxygen consumed by organic matter	0·405
Nitrogen as nitrates and nitrites	0·015
Total combined nitrogen	0·064
Chlorine	0·8
Temporary hardness	0·0
Permanent „	3·1
Total „	3·1

The sample was turbid, yellow-brown in colour, possessed a peaty taste, and contained animalcules visible to the naked eye.

One part of this peaty water was sterilised by steaming, and another part by filtration through a porous cylinder (Chamberland filter), and each of these sterilised waters was divided into two parts, which were respectively infected with the typhoid bacillus and with the *B. coli communis* on 30.10.1893, thus:—

Agar cultivations of the typhoid bacillus and of the *B. coli communis*, each twenty-seven days old and grown at 18—20° C., were employed:—

Twenty needle-loops of the typhoid growth were thoroughly shaken up with 25 c.c. of sterilised water.

Ten needle-loops of the growth of the *B. coli communis* were similarly shaken up with another 25 c.c. of sterilised water.

The peaty water was infected with the typhoid bacillus by adding 3 c.c. of the above water attenuation to 300 c.c. of the sterilised peaty water, whilst for infection with the *B. coli communis* only 1 c.c. of the water attenuation was added to 300 c.c. of the sterilised peaty water. The infected waters were placed in flasks plugged with cotton wool, and preserved in a dark cupboard at 10—12° C.

The fate of the bacilli (typhoid and coli) in this infected peaty water was then ascertained by means of periodical plate cultivations, which yielded the following results:—

Steam-sterilised Peaty Water, infected 30.10.1893 with—

Typhoid bacillus.	Plate cultivations made	*B. coli communis.*
	30.10.1893	
1776 colonies from 1 c.c. (plates incubated 6 days).		13,321 colonies from 1 c.c. (plates incubated 4—6 days).

Steam-sterilised Peaty Water, infected 30.10.1893 with—*continued.*

Typhoid bacillus.	Plate cultivations made	*B. coli communis.*
	6.11.1893	
1044 colonies from 1 c.c. (plates incubated 7 days).		14,031 colonies from 1 c.c. (plates incubated 7 days).
	15.11.1893	
432 colonies from 1 c.c. (plates incubated 5 days).		17,580 colonies from 1 c.c. (plates incubated 5 days).
	23.11.1893	
220 colonies from 1 c.c. (plates incubated 6 days).		14,457 colonies from 1 c.c. (plates incubated 4 days).

Peaty Water Sterilised by Filtration, infected 30.10.1893, with—

Typhoid bacillus.	Plate cultivations made	*B. coli communis.*
	30.10.1893	
1169 colonies from 1 c.c. (plates incubated 6 days).		21,603 colonies from 1 c.c. (plates incubated 4—6 days).
	6.11.1893	
No colonies on plates (plates incubated 9 days).		No colonies on plates (plates incubated 9 days).

Thus even in this water, heavily charged as it was with vegetable organic matter, the typhoid bacilli failed to undergo any multiplication, but, on the contrary, as usual, suffered a continuous numerical decline. The B. coli communis, *on the other hand, remained practically unaltered in numbers during the period of upwards of three weeks over which these observations extended, the slight numerical increase being too insignificant to be regarded as evidence of true multiplication.*

In the peaty water which had been sterilised by filtration through porous porcelain there was again the same phenomenon, so frequently referred to before in this Report, of the extraordinarily rapid disappearance of the introduced typhoid and coli bacilli.

On the possible Adaptation of the Typhoid Bacillus to active life in Potable Water.

In none of the experiments recorded above was any multiplication of the typhoid bacillus observed, although these experiments have been made with waters varying from the deep well water of the Kent Company, which is almost wholly destitute of organic matter, to the peaty water referred to on p. 537, which contains about the maximum amount of organic matter met with in water used for drinking purposes.

Some previous observers, on the other hand, record the multiplication of the typhoid bacillus in potable waters with which they have made similar experiments; whilst others, again, have found no multiplication. It appears to me highly probable that in most, if not in all, cases in which multiplication has been observed, it has been occasioned through the introduction of an appreciable amount of food-material along with the typhoid bacilli; for, as already pointed out, most investigators have exercised very little care in respect of this highly important factor.

But although the typhoid bacilli taken directly from an ordinary cultivation and plunged into potable water may not be able to proliferate in the latter, it appeared to me quite possible that if the environment of the typhoid bacilli were gradually, instead of suddenly, changed, the requirements of the bacilli might perhaps be thereby so far modified as to undergo multiplication in the aqueous medium. For by gradually changing the surroundings, it would be anticipated that those individuals most capable of flourishing under the altered conditions would be propagated, and that each successive generation of typhoid bacilli would thus become more adapted to the new medium.

To ascertain whether this process of education could be actually accomplished, the following experiments were made.

Education of Typhoid Bacilli for Aquatic Life.

A gelatine-culture of the typhoid bacillus was, in the first instance, inoculated into sterile broth of the ordinary strength, and kept at 18—20° C.; turbidity ensued in twenty-four hours. From this broth cultivation an inoculation was made into 50 per cent. broth (broth mixed with its own volume of water); this also became turbid in twenty-four hours at 18—20° C. From the 50 per cent. broth cultivation an inoculation was made into 10 per cent. broth (1 volume of broth mixed with 9 volumes of water); this liquid only became visibly turbid in from two and a half to three days. After four successive generations of cultivation in this 10 per cent. broth medium had been carried on, the time elapsing between the inoculation and the appearance of turbidity gradually diminished until, with the fifth generation, turbidity already set in in twenty-four hours. From this 10 per cent. broth, inoculation was then made into 1 per cent. broth (1 volume of broth mixed with 99 volumes of water); this became turbid in twenty-four hours. Continuous cultivation in this 1 per cent. broth medium was then carried on for a period of two months, after which it was employed for infecting steam-sterilised Thames water, thus:—

On 31.1.1894, 2 drops of a 1 per cent. broth cultivation (three

days old) of the typhoid bacillus were added to 600 c.c. of steam-sterilised Thames water, which was, on the day of infection and on several subsequent occasions, submitted to plate cultivation with the following results:—

Dates on which plates were prepared.	Number of days plates were incubated.	Number of typhoid bacilli in 1 c.c. of water.
31.1.1894	8	4,895
2.2.1894	7	15,372
5.2.1894	10	11,184
12.2.1894	7	6,558
23.2.1894	7	5,795
6.3.1894	6	6,068
10.3.1894	9	4,093

These figures show that unquestionable, although not very extensive, multiplication of the typhoid bacilli took place in the water thus infected; but in order to ascertain whether this proliferation was effected at the expense of the very small quantity of culture-material necessarily introduced along with the bacilli, or at the expense of the organic matter pertaining to the Thames water itself, the following further experiment was made:—

On 23.2.1894, 10 c.c. of the above-infected water, which on that day contained 5795 typhoid bacilli per 1 c.c., were added to 20 c.c. steam-sterilised Thames water, and the latter was then and several times subsequently submitted to plate cultivation with the following results:—

Dates on which plates were prepared.	Number of days plates were incubated.	Number of typhoid bacilli in 1 c.c. of water.
23.2.1894	7	1830
26.2.1894	7	1842
2.3.1894	6	375
6.3.1894	6	268

From these figures it is equally evident that in this case no multiplication but only numerical decline of the typhoid bacilli took place. If, however, the typhoid bacilli can proliferate at the expense of the organic matter belonging to the Thames water, they should have multiplied in the above experiment, as they were imported into a quantity of water, the organic matter of which had not since sterilisation been exposed to bacterial life; but from the fact that they did not multiply, but, on the contrary, only fell off in numbers, it becomes almost certain that the distinct multiplication observed in the former experiment was effected at the expense of the small quantity of food-material originally introduced into the water along with the typhoid bacilli.

Another experiment was made on similar lines to the first half of the above experiment, to see whether the multiplication there observed could be confirmed, thus :—

On 27.3.1894, 4 c.c. of a 1 per cent. broth cultivation (three days old) of the typhoid bacillus were put into 50 c.c. of steam-sterilised Thames water, the mixture being violently shaken up for fifteen minutes; 2 c.c. of this mixture (equivalent to $\frac{1}{27}$ c.c. of the original 1 per cent. broth culture) were then added to 200 c.c. of steam-sterilised Thames water, which was then submitted to plate cultivation as follows :—

Dates on which plates were prepared.	Number of days plates were incubated.	Number of typhoid bacilli in 1 c.c. of water.
27.3.1894	6	37,515
29.3.1894	6	61,566
31.3.1894	9	50,935
4.4.1894	6	27,818
11.4.1894	8	20,130

In this case again, therefore, there was a small but distinct multiplication.

From these experiments it appears that typhoid bacilli which have undergone a prolonged and gradual training in more and more aqueous culture-media do exhibit distinctly more vitality in potable water than bacilli which are at once transferred into water from highly nutritive solid media like agar or peptone jelly. On the other hand, there is considerable reason for believing that the slight but distinct multiplication which these trained bacilli undergo in potable water, is effected at the expense of small quantities of food-material introduced along with them at the time of infection, and not at the expense of the organic matter belonging to the water itself.

The result of the experiments with these specially-trained typhoid bacilli greatly fortifies the opinion which I have expressed above, that the extensive multiplication of typhoid bacilli in potable waters which has been observed by some investigators was most probably due to the importation of appreciable quantities of food-material along with the bacilli themselves.

Summary.

The investigation which has been detailed in the foregoing pages is divisible into the following sections :—

1. A series of experiments in which the vitality of one and the same culture of the typhoid bacillus was observed in one and the same sample of Thames water, using the latter under the following conditions :—

(a) In its natural unsterile state;
(b) Sterilised by steam;
(c) Sterilised by filtration through porous porcelain.

In each case the effect of temperature was studied by preserving the infected waters at winter and summer temperatures respectively.

(Description of experiments, see pp. 409—433, 451—465; summary of conclusions, pp. 433, 434.)

2. A perfectly similar series of experiments, carried on side by side with the above, in which the *Bacillus coli communis* was employed instead of the typhoid bacillus.

(Description of experiments, see pp. 409—433; summary of conclusions, pp. 433, 434.)

3. A series of experiments in which the effect of the addition of common salt in various proportions to unsterile Thames water was studied, the unsterile Thames water being employed for this purpose both uninfected and infected with the typhoid bacillus.

(Description of experiments, see pp. 434—450; summary of conclusions, p. 450.)

4. A series of experiments perfectly similar to No. 1 above, in which Loch Katrine water was employed instead of Thames water.

(Description of experiments, see pp. 465 *et seq.*; summary of conclusions, pp. 476—486.)

5. A further series of experiments made with the same sample of Loch Katrine water, only introducing a much larger number of typhoid bacilli into a given volume of water; in this series of experiments only unsterilised Loch Katrine water was employed.

(Description of experiments, see p. 486; summary of conclusions, p. 492.)

6. In order to compare the relative longevity of the typhoid bacillus in the more important types of potable water, a long series of experiments was carried out in which typhoid bacilli from one and the same cultivation, and in as far as possible equal numbers, were introduced into Thames water, Loch Katrine water, and the deep well water of the Kent Company respectively. Each of these waters was employed:—

(a) In its unsterile natural condition;
(b) Sterilised by steam;
(c) Sterilised by filtration through a porous cylinder of infusorial earth.

In this series of experiments the influence of rest or agitation was also studied.

(Description of experiments, see pp. 493 *et seq.*; summary of conclusions, pp. 516—518.)

7. A series of experiments made in order to ascertain whether the bactericidal properties of unsterilised surface water can be artificially induced by inoculating steam-sterilised Thames water with a few drops of unsterilised Thames water, and thus giving rise to a bacterial population in the previously sterile water.

(Description of experiments, see pp. 519 *et seq.*; summary of conclusions, pp. 528—530.)

8. Further experiments on the addition of common salt to typhoid-infected Thames water, both sterile and unsterile, with a view to confirming or contradicting the results of the experiments referred to under No. 3 above.

(Description of experiments, see pp. 530 *et seq.*; summary of conclusions, pp. 535—536.)

9. Experiments made to ascertain whether the typhoid bacillus and the *Bacillus coli communis*, multiply in potable water which is very highly charged with vegetable matter (peaty, upland, surface water).

(Description of experiments, see pp. 536 *et seq.*; summary of conclusions, p. 538.)

10. Experiments made to ascertain whether the typhoid bacillus can, by prolonged preliminary culture in more and more diluted media, be trained for aquatic life in potable water.

(Description of experiments, see pp. 539 *et seq.*; summary of conclusions, p. 541.)

For the detailed conclusions arrived at from the results of these several series of experiments, the reader is referred to the summaries which are appended to the descriptions of each series of experiments, as indicated above, whilst the general conclusions which arise out of the entire investigation may be summarised as follows :—

Summary of Conclusions.

1. Typhoid bacilli from ordinary agar-agar- and gelatine-cultures on being introduced into steam-sterilised potable water in such numbers as not to materially alter the composition of the latter undergo no multiplication. This result was uniformly obtained irrespectively of whether surface water like that of the Thames, which has received the drainage of manured land, or upland surface water like that of Loch Katrine, the organic matter in which is very similar in absolute

amount to that in Thames water, but almost exclusively derived from vegetable sources (peat); or, again, other upland surface water much more highly impregnated with peaty matter; or, lastly, deep well water containing the merest traces of organic matter, was employed.

In all cases, of course, special precautions were taken to prevent, as far as possible, the importation of culture-material along with the bacilli.

2. By first submitting the typhoid bacilli to prolonged culture in more and more aqueous media, and then introducing them into steam-sterilised Thames water, slight but distinct multiplication of the typhoid bacilli was observed, and, although, perhaps, by this method of training the typhoid bacilli had become more adapted to aquatic life, it appears probable that the multiplication observed took place at the expense of the minute quantity of culture-material necessarily introduced with them; for on transferring some of this infected water in which multiplication had taken place to a larger volume of the same steam-sterilised Thames water, no further multiplication was found to occur, showing that the organic matter belonging to the steam-sterilised Thames water itself was not capable of ministering to the growth and proliferation even of these specially educated typhoid bacilli.

3. Although no instance of multiplication of the introduced typhoid bacilli in these steam-sterilised potable waters was observed, on the other hand the bacilli were found to be possessed of very considerable longevity in them, thus:—

Description of steam-sterilised water.	Duration of life of typhoid bacillus.	
Thames water (11.5.1893) kept at 6—8° C.	Upwards of 76 days	Still just recognisable.
" " " 19° C.	" "	
Loch Katrine water (4.7.1893) kept at 6—8° C.	Upwards of 21 days	Only a small number of typhoid bacilli was introduced into these waters.
Loch Katrine water (4.7.1893) kept at 19° C.	Between 13 and 17 days	
Thames water (19.10.1893) kept at 9—12° C.	Between 32 and 39 days	Typhoid bacilli from one and the same source and in the same numbers were introduced into each of these waters.
Loch Katrine water (19.10.1893) kept at 9—12° C.	Upwards of 51 days	
Deep well water (19.10.1893) kept at 9—12° C.	Between 20 and 32 days	
Thames water (16.1.1894) kept at 9—12° C.	Upwards of 48 days (still abundantly present).	
Peaty upland surface water (17.10.1893) kept at 9—12° C.	Upwards of 24 days (still abundantly present).	

In no case was the duration of vitality a very limited one; its exact length in any particular water is doubtless dependent on the initial vitality of the bacilli and the numbers in which they are introduced. In the strictly comparative experiment on steam-sterilised Thames, Loch Katrine, and deep well water, it is seen that the longevity of the typhoid bacillus is distinctly greatest in the Loch

Katrine, and least in the deep well water, and intermediate between the two in Thames water. Of these three waters, also, the Loch Katrine contains the most, the deep well the least, and the Thames an intermediate amount of organic matter. Not improbably these circumstances are connected together.

4. The experiments distinctly show that in these steam-sterilised potable waters a summer temperature of 19° C. is more prejudicial than a winter temperature of 6—8° C. to the duration of life of the typhoid bacillus.

5. Inasmuch as the numerical estimation of typhoid bacilli in unsterile potable waters is practically impossible, the duration of life of the typhoid bacilli in such waters has alone been made the subject of study. This enquiry has involved an enormous amount of labour, as the certain detection, by means of the special methods employed, of the typhoid bacillus, even in a single specimen of water, may entail work extending over several weeks. The duration of life of the typhoid bacilli introduced into the various unsterile waters in the several series of experiments was as follows :—

Description of unsterile water.	Duration of life of typhoid bacillus.	
Thames water (11.5.1893) kept at 6—8° C.	Between 25 and 34 days.	
" " " 19° C.		
Loch Katrine water (4.7.1893) kept at 6—8° C.	Upwards of 17 days	Only a small number of typhoid bacilli was introduced into these waters.
Loch Katrine water (4.7.1893) kept at 19° C.	Between 4 and 11 days	
Loch Katrine water (7.7.1893) kept at 6—8° C.	Upwards of 14 days, after which no further examinations were made. (A much larger number of typhoid bacilli was introduced in this than in the above experiments.)	
Loch Katrine water (7.7.1893) kept at 19° C.		
Thames water (19.10.1893) kept at 9—12° C.	Between 9 and 13 days	Typhoid bacilli from one and the same source and in the same numbers were introduced into each of these waters.
Loch Katrine water (19.10.1893) kept at 9—12° C.	Between 19 and 33 days	
Deep well water (19.10.1893) kept at 9—12° C.	Between 33 and 39 days	
Thames water (16.1.1894) kept at 9—12° C.	Between 20 and 27 days.	

On comparing this table with that given under No. 3 above, it will be seen that in all cases, excepting one, the duration of life of the typhoid bacillus was greater, and often much greater, in the steam-sterilised than in the corresponding waters unsterilised. The single exception to this general rule was in the case of the deep well water in which the typhoid bacilli lived about the same length of time, irrespectively of whether the water was sterile or not.

The table also shows that, as in the case of the steam-sterilised waters, the exact length of time that the typhoid bacilli endured residence in one and the same type of unsterilised water was subject

to great variations in the different experiments, doubtless largely in consequence of the different initial vitality of the typhoid bacilli employed, and also, doubtless, in consequence of the different numbers in which they were introduced in the several series of experiments.

Of principal interest is the comparative experiment made with Thames, Loch Katrine, and deep well water, in which typhoid bacilli from one and the same source, and in the same numbers, were introduced into these three types of water, and in which the duration of life was found to be shortest in the Thames water (9—13 days), longest in the deep well water (33—39 days), and intermediate in the Loch Katrine water (19—33 days). This result is of very great practical importance as indicating the greater danger of typhoid bacilli gaining access to deep well than to surface water. This danger is, in actual practice, further enhanced by the fact that well water is almost invariably consumed without storage, whilst surface-waters are often stored for days or weeks, and in the case of upland surface water the storage frequently extends over many months.

The effect of temperature on the duration of life of the typhoid bacillus was well illustrated in the series of experiments with Loch Katrine water (4.7.1893), in which it was found that at 19° C. the typhoid bacilli had already disappeared in 4 to 11 days, whilst at 6—8° C. they were alive for upwards of 17 days in the same water.

The effect of agitation or rest on the typhoid bacilli in these waters was not very pronounced, but the evidence on the whole, both in the case of the sterilised and unsterilised waters, goes to show that the agitation or aëration of the water is unfavourable to the typhoid bacilli, and that in the unsterilised waters it occasions a more rapid multiplication of the water bacteria.

6. The greater bactericidal power of unsterilised than steam-sterilised surface waters is not apparently due to the multiplication of the water bacteria in the unsterile waters, bringing about a competition or "struggle for existence" between these aquatic forms and the typhoid bacilli, but rather to the elaboration of products by these aquatic bacteria (and very possibly also by other vegetable life present in surface waters) which are inimical and prejudicial to the welfare of the typhoid bacilli.

Thus, in the typhoid-infected unsterilised deep well water an enormously greater multiplication of the common water bacteria took place than in the unsterile Thames and Loch Katrine waters; yet, notwithstanding the typhoid bacilli not only lived much longer in this unsterile deep well water than in the unsterile Thames and Loch Katrine waters, but there was practically no difference between the duration of life of the typhoid bacilli in the sterile and unsterile deep well water respectively.

Of course it may be urged that the unsterile deep well water possibly does not contain those water bacteria which are particularly fitted for entering into successful competition with the typhoid bacilli, and that perhaps such water bacteria are only to be found in the unsterile surface waters.

7. The series of experiments summarised in the following table show that unsterile surface water, like that of the Thames, possesses bactericidal powers irrespectively of any further multiplication of its contained water bacteria, thus:—

Uninfected unsterilised Thames water, kept at 9—12° C., exhibited but little change in the number of its contained bacteria over the period of five weeks from 16.1.1894 to 24.2.1894. (The numbers only varied from 5500—2825 per 1 c.c.)

The same unsterilised Thames water, infected with about 170,000 typhoid bacilli per 1 c.c., exhibited a continuous decline in the total number of bacteria present in it over the same period.

The same Thames water, after sterilisation by steam, was infected with upwards of 100,000 typhoid bacilli per 1 c.c., and at the end of the same period (16.1.1894—24.2.1894) there were still upwards of 5000 typhoid bacilli per 1 c.c. present.

The same typhoid-infected steam-sterilised water was inoculated with a few drops of unsterile Thames water to communicate to it the Thames-water bacteria, and the latter underwent very extensive multiplication in this water. Notwithstanding, the typhoid bacilli lived between thirty-four and forty-one days in this water, whilst in the unsterile Thames water, in which no multiplication of the water bacteria took place, they only lived bet ·.en twenty and twenty-seven days.

This shorter duration of life of the typhoid bacilli in naturally unsterile Thames water than in that rendered unsterile by inoculation I attribute to the circumstance that in the naturally unsterile Thames water countless generations of water bacteria must have flourished before the water is made the subject of experiment at all, and it must, therefore, be more or less saturated with those bacterial products which are prejudicial to the vitality of the typhoid bacillus, and which, in fact, frequently hamper or even inhibit the further multiplication of the water bacteria themselves.

Thus it is obvious that the unsterile water in question was already, at the outset of the experiment, in such a condition as to prevent any multiplication of its own water bacteria, whilst, after it had been steam sterilised, the same bacteria multiplied abundantly in it. But again, at the outset of the experiment the unsterile water was in such a condition as to cause a comparatively rapid disappearance of the introduced typhoid bacilli, whilst after steam sterilisation it only became again endowed with this power of destroying the typhoid

bacilli when the introduced water bacteria had undergone extensive multiplication.

8. The addition of common salt to unsterile Thames water, in the proportion of 0·1, 1, and 3 per cent., causes the enormous multiplication of the water bacteria present, the most striking result in this respect being obtained with the largest addition of salt.

9. The addition of common salt to typhoid-infected unsterile Thames water diminishes the duration of life of the typhoid bacilli in this water, thus:—

	Duration of life of typhoid bacillus.
Unsterile Thames water kept at 6—8° C.	Between 25 and 34 days.
,, ,, ,, 19° C.	
Ditto with 0·1 per cent. salt, kept at 6—8° C.	,, 25 ,, 33 ,,
,, ,, ,, 19° C.	Less than 18 days.
Ditto with 1 per cent. salt, kept at 6—8° C.	,, ,,
,, ,, ,, 19° C.	,, ,,
Ditto with 3 per cent. salt, kept at 6—8° C.	,, ,,
,, ,, ,, 18° C.	,, ,,

10. This more rapid disappearance of the typhoid bacilli in the unsterile Thames water to which salt was added cannot be wholly, but only in part, attributed to the resulting multiplication of the water bacteria, as the addition of salt in similar proportion to typhoid-infected steam-sterilised Thames water also caused an exceedingly rapid disappearance of the typhoid bacilli, although the disappearance was not so rapid as in the same water to which a few drops of unsterile Thames water had been added, and in which, therefore, a great multiplication of the water bacteria took place, thus:—

	Duration of life of typhoid bacilli.
Steam-sterilised Thames water with 1 per cent. salt, kept at 9—12° C.	Between 12 and 19 days.
Ditto, to which also a few drops of unsterile Thames water were added, and in which extensive multiplication of the so-introduced water bacteria took place	Less than 5 days.

11. The *Bacillus coli communis*, taken from ordinary agar-agar cultures and introduced into steam-sterilised Thames water, undergoes considerable multiplication, when under precisely similar conditions the typhoid bacillus does not multiply.

The behaviour of the *B. coli communis* in the steam-sterilised waters may be summarised as follows:—

Description of steam-sterilised water.	Duration of life of *B. coli communis.*
Thames water (11.5.1893) kept at 6—8° C. Thames water (11.5.1893) kept at 19° C.	Still abundantly present, after considerable multiplication, on the 75th day.
Loch Katrine water (4.7.1893) kept at 6—8° C. Loch Katrine water (4.7.1893) kept at 19° C.	Between 14 and 17 days. The coli bacilli were introduced in this case in much smaller numbers than in that of the Thames water above. No multiplication was observed.
Very peaty water (30.10.1893) kept at 9—12° C.	Upwards of 24 days. Still present in undiminished numbers after very slight multiplication.

12. The *Bacillus coli communis* introduced into unsterile water persists in the living state for a much longer period than the typhoid bacillus. Thus:—

	Duration of life of the *B. coli communis.*
Unsterile Thames water (11.5.1893) kept at 6—8° C. Unsterile Thames water (11.5.1893) kept at 19° C.	Upwards of 40 days, and doubtless much longer, but no further examinations made.
Unsterile Loch Katrine water (4.7.1893) kept at 6—8° C. Unsterile Loch Katrine water (4.7.1893) kept at 19° C.	Upwards of 17 days. No further examinations made.

13. In the numerous experiments made on the behaviour of the typhoid bacillus and of the *B. coli communis* in water (Thames, Loch Katrine, deep well, and peaty water) which had been sterilised by filtration through cylinders of porous earthenware and baked infusorial earth, a most remarkably rapid disappearance of both bacilli was observed in all cases, excepting that of the Loch Katrine water. Further experiments will have to be made before any definite conclusions can be drawn from these unexpected results.

Appendix.

The Behaviour in Potable Water of Anthrax Bacilli taken directly from the Animal Body.

By Percy Frankland, Ph.D., B.Sc., F.R.S., and Charles Templeman, M.D., B.Sc.

In the 2nd Report to the Royal Society Water Research Committee, "On the Vitality and Virulence of the *Bacillus anthracis* and its Spores in Potable Water," the enquiry was mainly confined to the deportment either of anthrax spores alone, or of such mixtures of bacilli and spores which are found in the usual cultivations of anthrax

on artificial media. It is, however, obviously a matter of great importance to ascertain how anthrax bacilli, entirely free from spores, as they are found in the tissues of animals which have succumbed to this disease, behave when they are introduced into potable water. There is the more urgency for this investigation, as it was pointed out in the introduction to the Report that the experiments previously undertaken by others in this direction have led to highly discordant results.

The experiments which we have made on this subject were incidental to another investigation, on which we propose reporting later on, but as these experiments should have been made in connection with the 2nd Report, had time and opportunity permitted, we are bringing them forward now to fill up without further delay the hiatus in that Report.

First Series of Experiments.

The spleen of a white mouse, dead of anthrax, was excised under the usual aseptic precautions, and transferred to a small sterile bottle containing 20 c.c. of sterilised tap water, in which it was completely broken up by bruising with a sterile glass rod. More sterile water about 50 c.c. in all, was added to the bottle, and the whole violently shaken for fifteen minutes, so as to ensure even distribution of the bacilli throughout the water. 10 c.c. of this water attenuation were then added to about 400 c.c. of steam-sterilised Dundee water and thoroughly mixed, after which this infected water was distributed amongst a number of sterile tubes plugged with cotton wool. This infected water was submitted to plate cultivation on the same day, with the following results:—

Tube 16	$\frac{5}{9}$ c.c. water	yielded	8,190*	anthrax colonies	per 1 c.c.
	$\frac{2}{9}$,,	,,	7,871	,,	,,
Tube 17	$\frac{10}{25}$,,	,,	8,235	,,	,,
	$\frac{4}{25}$,,	,,	8,325	,,	,,
Tube 18	$\frac{5}{10}$,,	,,	11,712	,,	,,
	$\frac{2}{10}$,,	,,	8,100	,,	,,
Tube 19	$\frac{5}{12}$,,	,,	10,248	,,	,,
	$\frac{2}{12}$,,	,,	12,810	,,	,,
Tube 20	$\frac{5}{10}$,,	,,	9,408	,,	,,
	$\frac{2}{10}$,,	,,	6,050	,,	,,
			90,949		
	Average......		9,095		

Thus, on the day of infection the water contained about 9000 anthrax bacilli per 1 c.c.

* All these plates were incubated for 5 days at 18—20° C.

The tubes containing this infected water were kept in a dark cupboard at 12° C., and submitted to plate cultivation at intervals, thus:—

Plate cultures prepared.	Number of tube.	Number of days plates were incubated at 18—20° C.	Volume of water used for plate cultivation.	Number of anthrax colonies per 1 c.c. of water.
			c.c.	
2 days after infection	19	9	$\frac{5}{10}$	6
			$\frac{2}{10}$	10
,,	20	,,	$\frac{5}{10}$	236
			$\frac{2}{10}$	135
5 days after infection	19	9	$\frac{5}{10}$	All the plates were free from anthrax.
			$\frac{2}{10}$	
,,	20	,,	$\frac{5}{10}$	
			$\frac{2}{10}$	

These tubes were again examined on two subsequent occasions and again yielded sterile plates.

Thus these anthrax bacilli, taken directly from the dead mouse and introduced into sterile Dundee water in such large numbers as 9000 *per* 1 *c.c. of water, all died within five days, the water being kept at* 12° C.

Second Series of Experiments.

In this series of experiments the anthrax bacilli from the dead animal were introduced both into steam-sterilised Thames and steam-sterilised Dundee water respectively, and these waters were preserved at different temperatures, in order to ascertain the influence of this factor on the result.

The spleen of a white mouse, which had died of anthrax twenty-eight hours after inoculation, was broken up with sterilised tap water in the same way as already described above, and with this water attenuation larger volumes of sterile Thames and Dundee waters respectively were infected, and these infected waters were then distributed amongst a number of sterile tubes plugged with cotton wool. On the day of infection some of these tubes were submitted to plate cultivation, in order to ascertain the number of anthrax bacilli introduced, thus:—

Dundee water	Tube No. 1	$\frac{5}{10}$ c.c. water yielded	4840	anthrax colonies per 1 c.c.
		$\frac{2}{10}$,, ,,	4860	,, ,,
	,, 2	$\frac{5}{11}$,, ,,	6897	,, ,,
		$\frac{2}{11}$,, ,,	7722	,, ,,
Thames water	,, 7	$\frac{5}{10}$,, ,,	6136	,, ,,
		$\frac{2}{10}$,, ,,	5875	,, ,,
	,, 8	$\frac{5}{12}$,, ,,	6480	,, ,,
		$\frac{2}{12}$,, ,,	6552	,, ,,

The average number of anthrax bacilli in these infected Thames and Dundee waters at the outset was, therefore, about 6000 per 1 c.c. Some of these tubes were then placed in a refrigerator at 5° C., others were kept in a cupboard at 13° C., whilst others were put in an incubator at 19° C. The water in these tubes, kept at the different temperatures specified, was submitted to plate cultivation at intervals, with the following results:—

Water kept at 5° C.

Plate cultures prepared.	Description of water.	Number of tube.	Number of days plates were incubated at 18—20° C.	Volume of water used for plate cultivation.	Number of anthrax colonies per 1 c.c. of water.
				c.c.	
1st day after infection	Thames	9	6	$\frac{5}{10}$	1028
				$\frac{2}{10}$	2470
		10	,,	$\frac{10}{25}$	2550
				$\frac{4}{25}$	2031
,,	Dundee	3	,,	$\frac{10}{19}$	3386
				$\frac{4}{19}$	3733
		4	,,	$\frac{5}{10}$	3456
				$\frac{2}{10}$	3335
2 days after infection	Thames	9	6	$\frac{5}{11}$	834
				$\frac{2}{11}$	660
		10	,,	$\frac{5}{10}$	860
				$\frac{2}{10}$	485
,,	Dundee	3	,,	$\frac{5}{10}$	1624
				$\frac{2}{10}$	1995
		4	,,	$\frac{10}{19}$	1864
				$\frac{4}{19}$	1497
5 days after infection	Thames	9	6	$\frac{5}{12}$	None
				$\frac{2}{12}$	,,
		10	,,	$\frac{5}{10}$	,,
				$\frac{2}{10}$	,,
,,	Dundee	3	,,	$\frac{10}{29}$	134
				$\frac{4}{29}$	235
		4	,,	$\frac{10}{31}$	None
				$\frac{4}{31}$	,,
14 days after infection	Thames	9	6	1·0	None
			,,	0·5	,,
		10	,,	1·0	,,
			,,	0·5	,,
,,	Dundee	3	,,	1·0	,,
			,,	0·5	,,
		4	,,	1·0	,,
			,,	0·5	,,

Thus, in the Thames water maintained at 5° C. the anthrax bacilli (introduced to the number of 6000 per 1 c.c.) had already undergone very

considerable diminution in numbers on the day following their introduction; after two days the numbers had still further diminished, whilst five days after introduction they were no longer discoverable at all. The fate of the anthrax bacilli in the Dundee water was quite similar, their disappearance being, however, a little less rapid; thus a few bacilli were still present in one of the two tubes on the fifth day after infection.

The following table exhibits the results obtained with the same waters kept at 13° C.:—

Water kept at 13° C.

Plate cultures prepared.	Description of water.	Number of tube.	Number of days plates were incubated at 18—20° C.	Volume of water used for plate cultivation.	Number of anthrax colonies per 1 c.c. of water.
				c.c.	
1st day after infection	Thames	7	6	$\frac{5}{10}$	2640
				$\frac{2}{10}$	2870
		8	,,	$\frac{5}{10}$	3564
				$\frac{2}{10}$	3505
,,	Dundee	1	,,	$\frac{5}{11}$	4422
				$\frac{2}{11}$	4048
		2	,,	$\frac{5}{9}$	3331
				$\frac{2}{9}$	3153
2 days after infection	Thames	7	6	$\frac{5}{10}$	1690
				$\frac{2}{10}$	—
		8	,,	$\frac{5}{10}$	2196
				$\frac{2}{10}$	2035
,,	Dundee	1	,,	$\frac{5}{9}$	2442
				$\frac{2}{9}$	2848
		2	,,	$\frac{5}{10}$	2806
				$\frac{2}{10}$	2785
5 days after infection	Thames	7	6	$\frac{5}{13}$	910
				$\frac{2}{13}$	—
		8	,,	$\frac{5}{10}$	1096
				$\frac{2}{10}$	1240
,,	Dundee	1	,,	$\frac{5}{11}$	—
				$\frac{2}{11}$	1427
		2	,,	$\frac{5}{10}$	2283
				$\frac{2}{10}$	1965
14 days after infection	Thames	7	6	1·0	None
			,,	0·5	,,
		8	,,	1·0	,,
			,,	0·5	,,
,,	Dundee	1	,,	1·0	,,
			,,	0·5	,,
		2	,,	1·0	,,
			,,	0·5	,,

Thus at the higher temperature of 13° C., the anthrax bacilli were markedly more persistent than at 5° C., although they had in all cases

become largely reduced in numbers by the fifth day after their introduction into the water, and by the fourteenth day they had all disappeared.

In the following table are recorded the results which were obtained with the same waters maintained throughout at a temperature of 19° C. :—

Water kept at 19° C.

Plate cultures prepared.	Description of water.	Number of tube.	Number of days plates were incubated at 18—20° C.	Volume of water used for plate cultivation.	Number of anthrax colonies per 1 c.c. of water.
				c.c.	
1st day after infection	Thames	11	6	$\frac{5}{10}$	3,400
				$\frac{2}{10}$	3,200
		12	,,	$\frac{5}{9}$	2,786
				$\frac{2}{9}$	2,830
,,	Dundee	5	,,	$\frac{5}{12}$	6,110
				$\frac{2}{12}$	4,908
		6	,,	$\frac{5}{12}$	5,327
				$\frac{2}{12}$	5,718
2 days after infection	Thames	11	6	$\frac{5}{10}$	3,530
				$\frac{2}{10}$	4,420
		12	,,	$\frac{5}{12}$	4,066
				$\frac{2}{12}$	4,253
,,	Dundee	5	,,	$\frac{10}{25}$	5,125
				$\frac{4}{25}$	10,000
		6	,,	$\frac{5}{10}$	5,200
				$\frac{2}{10}$	5,745
5 days after infection	Thames	11	6	$\frac{10}{19}$	30,428
				$\frac{4}{19}$	36,020
		12	,,	$\frac{5}{10}$	32,448
				$\frac{2}{10}$	28,194
,,	Dundee	5	,,	$\frac{5}{10}$	46,200
				$\frac{2}{10}$	45,825
		6	,,	$\frac{5}{12}$	32,370
				$\frac{2}{12}$	25,140
14 days after infection	Thames	11	6	$\frac{10}{27}$	—
				$\frac{4}{27}$	207,238
		12	,,	$\frac{10}{25}$	—
				$\frac{4}{25}$	163,750
,,	Dundee	5	,,	$\frac{10}{25}$	—
				$\frac{4}{25}$	66,581
		6	,,	$\frac{5}{12}$	—
				$\frac{2}{12}$	54,300
42 days after infection	Thames	11	3	$\frac{10}{25}$	—
				$\frac{4}{25}$	99,937
		12	,,	$\frac{5}{13}$	109,590
				$\frac{2}{13}$	102,180
,,	Dundee	5	,,	$\frac{5}{10}$	—
				$\frac{2}{10}$	66,240
		6	,,	$\frac{5}{12}$	51,227
				$\frac{2}{12}$	49,610

Thus at the temperature of 19° C., the behaviour of the anthrax bacilli in these same waters was entirely different; far from their undergoing rapid diminution in numbers followed by early disappearance, they only exhibited slight diminution during the first few days after their introduction, upon which there followed an enormous multiplication. This multiplication was already observable, in the case of one tube, on the second day after infection, whilst on the fifth day it was very pronounced in all the tubes, and on the fourteenth day the numbers reached were very large, remaining practically unaltered even on the forty-second day.

The explanation which naturally suggests itself for this entirely different behaviour of the anthrax bacilli at the higher and the lower temperature respectively, is that at the higher temperature of 19° C. the anthrax bacilli can form spores, whilst at the lower temperatures this sporulation cannot take place. With the appearance of the spores, however, the longevity of anthrax in sterile potable water becomes, as was shown in the Second Report, practically indefinite.

In order to test the validity of this hypothesis that sporulation had taken place in the waters kept at 19° C., the following experiments were made:—

(1.) 1 c.c. of the contents of Tube 11 (Thames water, see table above) was kept at 70° C. for ten minutes, in order to destroy anthrax bacilli; on subsequent plate cultivation, innumerable anthrax colonies were obtained.

(2.) A similar experiment made with 1 c.c. of the contents of Tube 12 (Thames water, see table above) gave exactly the same result.

(3.) A similar experiment made with 1 c.c. of the contents of Tube 5 (Dundee water, see table above) gave the same result, innumerable anthrax colonies being obtained on the plate.

(4.) A similar experiment made with 1 c.c. of the contents of Tube 6 (Dundee water, see table above) gave 23,352 anthrax colonies.

Thus in the case of all these waters kept at 19° C., it is evident that practically the whole of the anthrax microbes present at the end of forty-two days were there in the condition of spores, showing as they did no appreciable diminution in numbers by the process of heating to 70° C. for ten minutes.

These experiments show, then, very clearly that the fate of virulent anthrax bacilli passing from an anthrax victim into potable water will be dependent on the temperature of the latter; if the temperature of the water is below that at which sporulation of anthrax can take place, then the bacilli will perish in the course of a few days; whilst if the temperature is high enough to admit of sporulation,

then anthrax spores will be formed, and these, as is now well known, may persist in a living and virulent condition for an almost indefinite period of time—for months and probably even for years.

From these experiments it further appears that the lowest temperature at which spore formation of anthrax in water will take place lies somewhere between 15° and 19° C. According to Koch, the lowest temperature at which anthrax spores are obtained is 16° C., and the most advantageous temperature for the production of the hardiest spores is 20—25° C.

FIG. 3.